Visual inspection plus Human Papillomavirus testing or Liquid-based Cytology: how best to control cervical cancer in Peru?

Thesis submitted for the degree of Doctor of Philosophy

by

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September 2003

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ABSTRACT

The main objective of this thesis was to estimate the sensitivity and specificity of four screening tests: visual inspection after the application of acetic acid (VIA), VIA_M: combined VIA and VIAM (magnified VIA using an AviScope™ performed by a doctor), liquid-based cytology (LBC using the AutoCyte-Prep® manual system) and HPV testing (using Hybrid Capture II); to detect histologically confirmed high-grade squamous intra-epithelial lesions (HSIL). Participants of the "Tamizaje y Tratamiento Inmediato de lesiones cervico-uterinas" (TATI) project, who signed an informed consent for additional cervical samples, were included in this study. Separate cervical samples for conventional cytology (CC), LBC and Hybrid Capture II (HC-II), were collected by a midwife before applying acetic acid (5%) to the cervix and performing VIA. Women testing positive on VIA were referred to VIAM performed by a doctor who confirmed the midwife diagnosis, and treated lesions with cryotherapy if appropriate, or referred women to colposcopy. Negative women on VIA_M; underwent colposcopy if they had HSIL on CC or LBC; or had second screening if they had any lesser abnormality on LBC or were positive only on HC-II. Of 5565 participants, 104 had histologically confirmed HSIL and an estimated 112 had undetected/unconfirmed HSIL. Sensitivities of VIA, VIA_M, LBC (any abnormality), LBC (high-grade abnormality) and HC-II were 44% (95% confidence interval (CI): 34,59), 31% (CI: 20,50), 69% (CI: 61,78), 35% (CI: 23,56) and 72% (CI: 65,79), respectively. VIA had the lowest specificity among all tests: 77% (CI: 74,77). LBC ≥HSIL had the highest PPV: 44% (CI: 34,53). HC-II was more sensitive than the other tests in our study. LBC ≥HSIL was more specific than the other tests. VIA had low sensitivity and specificity. VIA could still be an option for developing countries if implemented with regular quality assurance.

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To my mother

ACKNOWLEDGMENTS

I am very grateful to Cancer Research UK for sponsoring this PhD. In particular, I would like to thank Professor Jack Cuzick for supporting my project since the beginning.

I want to thank my supervisor Martin Bobak, for all the support and encouragement needed to keep going, for all the writing recommendations and constant advice.

My special thanks to my other supervisor Peter Sasieni, for supporting me throughout these four years, being patient, extremely helpful, friendly; for all his comments, suggestions and recommendations. Under his supervision, my knowledge has increased exponentially in epidemiology and statistics.

I am grateful to PAHO and PATH, who allowed me to incorporate my study into their TATI intervention project; and to the Ministry of Health of Peru and DIRES for supporting the TATI project, and my study. Many thanks to everybody in the TATI office; and to the women that participated in this study.

I am grateful to the "Maes-Heller" Cancer Research Centre and to the Peruvian Cancer Foundation, for their support, especially to Dr. Eduardo Caceres Graziani, for his invaluable help to put into practice this study and for his constant encouragement to finish and publish this thesis; and to Yesi for all her help with the samples. Many thanks to doctors Rina Takahashi and Juvenal Sanchez, for their great job and to everyone in the cytology laboratory at INEN.

Special thanks to Linda, George and Philip for their fantastic job, without them I would have no results.

Special thanks to Joanna Adams for her help and company in crucial thesis moments and to everyone in the EMS department for being nice to work with.

GLOSSARY

AAIR: Age-adjusted incidence rate.

ASCUS: Atypical squamous cells of undetermined significance.

AGUS: Atypical glandular cells of undetermined significance.

CI: 95% Confidence Interval.

CIN: Cervical intraepithelial neoplasia.

CR: Cumulative rate up to age 74.

C.S.: Centro de Salud. Health centre.

DIRES: Direccion Regional de Salud San Martin. Health Directive Office of the San Martin region of Peru.

HPV: Human papillomavirus

H.R.: Hospital Rural. Rural hospital.

HSIL: High-grade squamous intraepithelial lesion.

INEN: Instituto de Enfermedades Neoplasicas. Cancer hospital in Lima.

LEEP: Loop electrosurgical excision procedure, sometimes used as LLETZ: Large loop electrosurgical excision of the transformation zone.

LBC: Liquid-based cytology

LSIL: Low-grade squamous intraepithelial lesion.

NICE: National Institute for Clinical Excellence.

PPV: Positive predictive value.

P.S.: Puesto de salud. Health post (small health centre).

RLU: Relative lights units.

SIL: Squamous intraepithelial neoplasia.

VIA: Visual inspection of the cervix after the application of acetic acid (nominally 5%).

VIAM: Magnified VIA or aided VIA using a magnification device.

1. INTRODUCTION

Cervical cancer is the second most common cancer among women worldwide [1], with almost half a million new cases and quarter of a million deaths each year [1]. The aetiology of cervical cancer is not fully understood but it is now accepted that Human Papillomavirus (HPV), most frequently HPV 16, is the most important etiological agent for cervical cancer [2]. The overall prevalence of HPV infection was estimated as 99.7% among cervical cancers worldwide, this gave HPV the highest attributable fraction ever identified for a specific cause of a major human cancer [3].

In terms of cervical cancer control, it has been well established that organised cytology screening programmes can substantially reduce the incidence and mortality from cervical carcinoma in developed countries. However, such programmes have not been successful in developing countries.

Study setting

The study is based in Peru, a country well suited to study cervical cancer and its control. Cervical cancer is the most common cancer among women in Peru, as reported by the only two cancer registries in the country: Trujillo and Lima. The incidence rate (age-standardise rate per 10^5 women) of cervical cancer for women in Trujillo was of 53.5 for the period 1988-90, while in Lima, it was 26.1 between 1990-1991 [4]. Data on cervical cancer aetiology in Peru are consistent with studies elsewhere. In a case-control study in Peru, the prevalence of HPV infection among cases was 94.9% and 17.7% among controls, indicating that HPV is the most important and probably a necessary cause of cervical cancer among Peruvian women [5]. Several attempts to establish a national screening programme in Peru have failed, but in 1998 the Ministry of Health formulated a National Plan for Gynaecological Cancer Prevention: Cervix and Breast and in 1999 declared cervical cancer a national priority.

Peru has special geographic characteristics. It is composed of three marked regions: coastal desert, mountains (the Andes) and rainforest (Amazon jungle), with extremely diverse climates, altitudes and cultures. These characteristics make the country propitious for research on cancer risk factors since life-styles and accessibility to health and other facilities differ greatly but at the same time a real challenge to establish any national screening programme.

Rationale for this study

Given the prominence of cervical cancer for women's health worldwide and in Peru, it is crucial to investigate strategies to control this disease. Screening has been found to be effective in western countries but much less so in developing countries. This project seeks to fill this gap. This study intends to evaluate the effectiveness of four different screening techniques, or a combination of them, in a province of the Amazonia of Peru. These techniques are (i) unaided visual inspection of the cervix after application of 5% acetic acid (VIA), (ii) a combination (VIA_M) of unaided VIA with aided visual inspection using a magnification device (VIAM), (iii) HPV DNA testing using Hybrid Capture II (HC-II), and liquid-based cytology (LBC) using the AutoCyte-Prep® manual system. The study is nested within a larger project called "Tamizaje y Tratamiento Inmediato de Lesiones Cervico-uterinas" (TATI), which aims to screen 80% of women in the region over three years. The success of the TATI intervention will be measured in terms of the number of women with successfully treated histologically confirmed high-grade disease.

Structure of the thesis

The next chapter of this thesis presents the background to the problem of cervical screening and the suggested strategy for its solution. It is divided upon three parts. The first part presents a brief description of the cervix and its clinical features, a short introduction into cervical cancer precursors and

invasive cancer, some epidemiological facts, risk factors starting with human papillomavirus infection (HPV) and its relationship to cervical neoplasia. The second part deals with prevention and control of cervical cancer. Screening is defined and the three screening tests used in this project are reviewed. The potential for new preventive and therapeutical cervical cancer vaccines is introduced. The last part of the chapter describes the problem of cervical cancer in Peru, i.e. the rates of disease and the efforts to control the burden of cervical cancer in the country. It also gives details of the "Tamizaje y Tratamiento Inmediato de Lesiones Cervico-uterinas" (TATI) project with which the current project is associated.

The third chapter presents the aim, the objectives and the hypothesis of the project. The objectives are divided in main and secondary objectives. As mentioned above, the aim of the project was to evaluate the sensitivity, specificity, acceptability and feasibility of various combinations of the screening techniques.

The fourth chapter describes the methodology. It presents the design of the study, divided into two parts, the statistical analysis and briefly the ethical considerations. In the first part, the details of screening procedures are listed and clinical management of VIA or VIAM positive women is explained. The second part deals with follow-up of initial screening and the management of untreated women who tested positive on HC-II or LBC. Laboratory techniques, sample handling and data management are also summarised. The statistical analysis starts with the definition of disease status. The statistical methods are then divided in descriptive statistics, estimation of missing screening test results, and estimation of sensitivity and specificity of screening tests or of combinations of them. Multinomial logistic modelling was used to estimate incomplete information regarding screening tests. Empirical imputation and logistic regression modelling were used to estimate disease status among those women who were not fully evaluated and weighted measures of efficacy were estimated.

Bootstrapping was used to estimate 95% confidence intervals (CI). Finally, sample size considerations and ethical considerations are addressed.

The fifth chapter presents the results. First, the numbers of women and the inclusion/exclusion criteria are presented. Demographic data such as age, education and screening centre distributions are tabulated. Reproductive and sexual factors are summarised and data on previous cytology are presented. Positivity rates of screening tests are also presented. Women are grouped into 6 different clinical management strata, which are summarised in six flowcharts. The number of women receiving different treatments, and their histology results are also summarised. Finally, estimates of sensitivity, specificity and positive predictive value, together with 95% CI are tabulated.

The fifth chapter is dedicated to discussion of the design and the results of the study. Topics covered include: participants, health providers and screening techniques used; the advantages and problems encountered with each screening test, and their positivity rates are compared to those of similar studies. Before examining sensitivity and specificity of each screening test and of some combinations of tests, the histology results obtained during the study are evaluated; a correspondent quality assessment is summarised. Then, the advantages of using a special information system and a basic cost-analysis are presented. Finally, but no less important, the future of cervical screening and the evaluation of new techniques, in Peru and worldwide are presented.

The seventh chapter presents the conclusions and recommendations based on the thesis.

2. BACKGROUND

2.1. Cervical cancer

2.1.1. Introduction

Cervical cancer is an important public health problem worldwide. It is the second most common cancer among women, ranking first in many developing countries [1]. Of 468,000 new cases and 233,000 deaths of invasive cervical cancer estimated for year 2000 [6], 80% occur in less developed countries where it disproportionately affects poor women who are the least likely to receive effective treatment, and so very prone to die of the disease.

It has been shown that effective screening can prevent cervical cancer, by detecting preinvasive lesions, which can be treated before becoming cancer.

In most developed countries, well-organised screening programmes have succeeded in reducing incidence and mortality from cervical cancer. A clear example is Finland, where there has been a significant reduction in incidence of cervical cancer: age-adjusted incidence rates of cervical cancer (AAIR per 100,000 women) fell from 15 to 5 (67% reduction) from 1961 to 1985 [7].

Unfortunately, in developing countries, where most of the burden of cervical cancer is concentrated, screening for cervical cancer is mainly opportunistic. Very few cervical lesions are detected, and they are usually in advanced incurable stages [8]. Women will not be informed of their results and detected lesions will not be treated [9,10].

2.1.2. Clinical Features

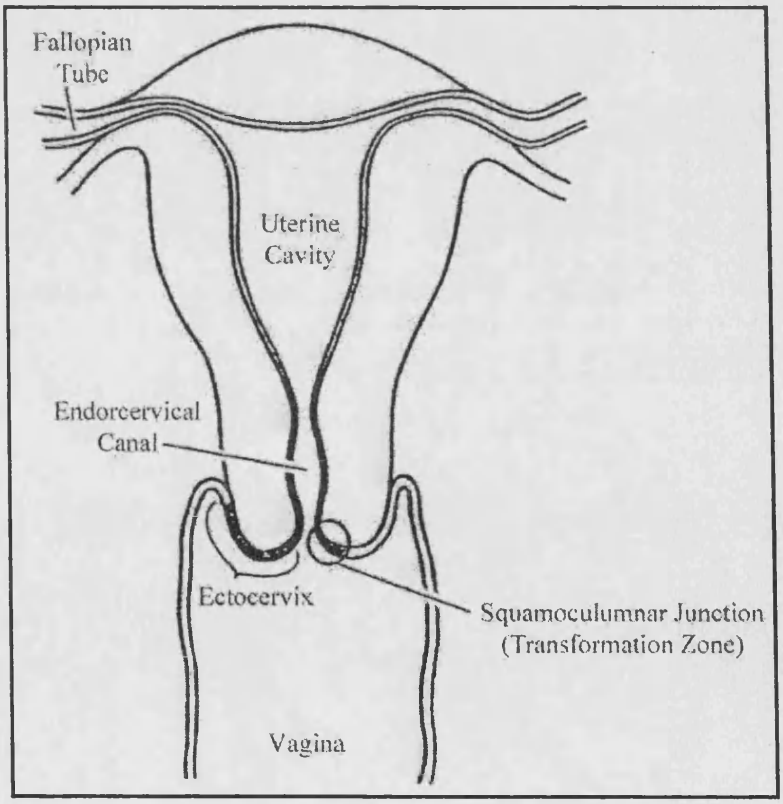
To understand the clinical features of cervical cancer, some basic knowledge about anatomy and physiology is necessary. The uterus is divided into two parts. The upper part, or body of the uterus is where a

fetus grows. The cervix is the part of the uterus below the internal os. The cervix connects the body of the uterus to the vagina (birth canal). The part of the cervix closest to the uterus is called the ***endocervix***. The part next to the vagina is the ***ectocervix*** or ***portio***. The endocervix is lined with mucous columnar epithelium, the ectocervix, with squamous epithelium. The transition zone between the two: the ***squamocolumnar junction (SCJ)*** is the area that is predisposed to malignant change.

2.1.2.1. The transformation zone

The SCJ of the cervix is the point at which the endocervical columnar epithelium meets the ectocervical stratified squamous epithelium. This junction is not at a fixed point on the cervix throughout life.

Figure 2.1.2.1.1. Diagram of the uterus and cervix.



At birth, the SCJ is said to be located at, or close to, the external os of the cervix. This point is often called the "original" SCJ. During puberty, because of ovarian hormonal production there is an increase in the size of both the corpus and cervix uterine. This leads to eversion of the cervix. As a consequence, the endocervical epithelium then lies on the vaginal portion of the cervix and is exposed to the acidic environment of the vagina. It seems to be mainly this stimulus that leads to ***squamous metaplasia***, a physiologic process in which a series of cell changes culminate in replacement of the everted endocervical columnar epithelium into matured squamous epithelium. The zone where the change of epithelium has occurred is called the ***transformation zone*** (see Figure 2.1.2.1.1).

When ovarian hormonal stimulation decreases at menopause, the eversion of the cervix is reversed and the SCJ passes into the endocervical canal [11].

As the majority of cervical cancers arise in this transformation zone, cryotherapy, laser surgery or loop electrical excision (LEEP) over it are the well-established treatment procedures when preinvasive lesions are found [11].

2.1.3. Intra-epithelial precursors of cancers

It has been generally recognised and accepted that precursors lesions to invasive squamous cervical cancer, comprise a morphologic and biologic spectrum of changes, from mild, moderate and severe dysplasia to carcinoma in situ (CIS). These four non-invasive categories are part of a single neoplastic continuum and have been conventionally graded according to progressive epithelial differentiation or "atypia of epithelial cells" and progressive involvement of the full thickness of the epithelium, from the basal layers in milder lesions, to the increasingly mature squamous cells as the mucosal surface is approached. Thus, in CIS, the full thickness of the epithelium is involved by undifferentiated cells, and indeed, any of these categories may coexist at different sites within the same cervix.

Mild dysplasia is also called cervical intraepithelial neoplasia one (CIN I), terminology introduced by Richart in 1973 [12], or as low-grade squamous intraepithelial (low-grade SIL) according to the Bethesda system developed in 1988 [13] and reviewed recently [14]. Table 2.1.3.1 presents the different terminology used for squamous cervical lesions.

The category low-grade SIL also includes the cytological diagnosis of koilocytotic atypia, which corresponds to cytological changes characteristic of infection with HPV [15].

Each of the preinvasive lesions has three options for further development: *(i)* regression, *(ii)* progression to the next higher step in the sequence of dysplasia until CIS, or *(iii)* development into invasive cancer. Several follow-up studies aiming to estimate rates of regression and progression have shown that the majority of low-grade lesions will regress to normal [15] and that the worse the abnormalities, the higher the likelihood of progression into higher lesions or invasive cancer [16,17]. Hence, the likelihood of development into invasive cancer is particularly high in severe dysplasia and CIS [18], and furthermore, 10 to 15% of untreated CIS will progress into invasive cancer within 3 to 5 years [19].

Table 2.1.3.1 Common classification of Cervical Squamous Neoplasia

CIN Scale	BSCC Scale[1]	Bethesda system
Koilocytotic or condylomatous atypia	Koilocytotic or condylomatous atypia	Low-grade SIL including koilocytosis
CIN I	Mild dysplasia	Low-grade SIL
CIN II	Moderate dysplasia	High-grade SIL
CIN III	Severe dysplasia/ carcinoma in situ	High-grade SIL
Invasive cancer	Invasive cancer	Invasive cancer

1: Advocated by the British Society for Clinical Cytology

2.1.4. Histopathology of invasive cervical cancer

There are three main histological types of cervical carcinomas: squamous, adeno- and adenosquamous carcinomas. The distribution of them has changed in recent years. In the past, between 85% and 90% of cervical carcinomas were squamous and most of the rest were adenocarcinomas. According to data from "Cancer Incidence in Five Continents Volume VIII" [20], in well-screened populations the percentage of adenocarcinomas has increased up to 28%, while in areas with no screening programmes, squamous carcinomas still account for about 80% to 90% of all cervical cancers. Other histological types, such as melanomas, sarcomas, and metastasic tumours, are very rare.

Several studies have reported increasing incidence rates of cervical adenocarcinomas over time, especially in young women in the United States [21,22,23,24], England and Wales [25,26], Norway [27] and Sweden [28]. Reasons for the increase are such as the increase in the prevalence of HPV infection and the improvement in screening, however, they are not definite [29].

Most epidemiological studies have focused on squamous carcinomas or have ignored histologic distinctions altogether [11].

2.1.5. Descriptive Epidemiology

Cervical cancer is the second most frequent cancer in women all over the world, after cancer of the breast and the most common in developing countries [1]. When men and women are considered together, cancer of the cervix is the seventh most common cancer worldwide, after cancers of the lung, breast, colon/rectum, stomach, liver and prostate, accounting for an estimated 4.7% of all cancers.

Table 2.1.6.1 Estimated number of cases and age standardised-incidence rates of cervical cancer by region of the world (2000).

	Cervical cancer	
Region	New cases	AAIR
Eastern Africa	30,206	44.3
Central America	21,596	40.3
The Caribbean	6,670	35.8
South America	49,025	30.9
Southern Africa	5,541	30.3
South Central Asia	151,297	26.5
Middle Africa	6,947	25.1
Western Africa	13,903	20.3
South Eastern Asia	39,648	18.3
Eastern Europe	35,482	16.8
Northern Africa	10,479	16.8
Western Europe	13,282	10.4
Southern Europe	10,116	10.2
Northern Europe	6,049	9.8
US/Canada	14,845	7.9
Australia/New Zealand	1,077	7.7
Eastern Asia	51,266	6.4
Western Asia (the Middle East)	3,458	4.8
World	470,606	16.1

AAIR = Age-adjusted incidence rates per 100,000 women.

Almost 80% of the cases of cervical cancer occur in developing countries. Incidence and mortality rates vary greatly within region of the world and within countries. Table 2.1.6.1 shows the number of new cases and the age-adjusted incidence rates per 100,000 women (AAIR) by region as estimated by GLOBOCAN [30] for the year 2000. The highest rates are observed in most of Africa, Latin America and South Central Asia.

Intermediate rates are observed South Eastern Asia, Northern Africa, and Eastern Europe, while the lowest rates are seen in the rest of Europe, the United States and Canada, Australia and New Zealand, Eastern Asia (mainly China) and the Middle East.

Table 2.1.6.2. shows the estimated incidence rates of some countries in Latin America and Africa. Haiti has the highest and most striking rate. Also Nicaragua and Bolivia have very high rates, and not much lower are those of most South American countries (AAIR over 30), higher than that of Romania (AAIR 31.5), ranking number one in Europe. The variation within Africa is also great, rates range from 61 in Tanzania to less than 7 in Tunisia.

Table 2.1.6.2. Incidence rates of cervical cancer in Latin America and Africa (2000)

Latin America	Cervical cancer		Africa	Cervical Cancer	
Country	AAIR	CR (%)	Country	AAIR	CR (%)
Haiti	93.9	4.9	Tanzania	61.4	4.6
Nicaragua	61.1	4.3	Zimbabwe	52.1	3.5
Bolivia	58.1	4.1	Rwanda	48.1	3.7
Ecuador	44.2	3.1	Gabon	31.7	2.2
Costa Rica	40.6	1.7	South Africa	28.9	2.0
Peru	40.0	2.9	The Gambia	26.0	2.0
Colombia	32.9	2.4	Algeria	23.4	2.0
Brazil	31.3	2.2	Sudan	19.0	1.3
Cuba	23.9	1.7	Cameroon	16.6	1.2
Uruguay	13.9	1.1	Egypt	14.0	1.1
Puerto Rico	10.3	0.7	Tunisia	6.8	0.5

AAIR = age-adjusted incidence rates. CR = cumulative rate to age 74.

There is also a lot of variation of incidence rates of cervical cancer both within and between regions and within and between countries, shown by

cancer registry reports. For instance, incidence rates among different ethnic groups in the United States vary from 7.3 (among white women) to 11.7 (among black women). This variation has been explained by socio-economical and education levels within developed countries.

It has been suggested that variations in the prevalence of HPV could explain part of the between and within countries variation of cervical cancer. Also, social factors, sexual behaviour and the impact of screening programmes have been proposed as potential reasons, but consistent results have not been achieved.

2.1.6. Risk factors for cervical cancer

The relationship between sexual behaviour and cervical cancer was postulated for the first time in 1842, when it was observed that frequency of cervical cancer among virgins and nuns was low [31]. Sexual transmitted diseases like Neisseria gonorrhoe, Trichomonas and Treponema species, Chlamydia and genital herpes, were then suggested as possible causes of cervical cancer but the main etiologic factor was yet to be discovered. Zur Hausen [32] was the first to relate human papillomaviruses (HPV) to cervical cancer. Since then, evidence coming from several studies has established that high-risk HPV types are the main causal factor of cervical cancer.

2.1.7. Human Papillomavirus infection

The Human Papillomavirus (HPV), is a member of the papovirus group, which typically causes benign, regressive squamous cell papillomata in the skin or mucosa in a large number of animal species [33] including human beings.

Genital infection with HPV is one of the most common sexually transmitted diseases, its prevalence in young women ranging from 20% to 46% in various countries [34,35,36,37,38,39].

Studies in Zur Hausen's laboratory were the first to relate HPV 6 and HPV 11 to human genital warts [32,40] and HPV 16 to cervical cancer [41], but it was

not until 1987 that Zur Hausen pointed out that HPV had a possible role in human genital cancer [42,43]. Since then, more than 35 distinct types of HPV have been recognized to infect the genital tract [44]. Twenty or more of these HPV types are cancer associated. Types 16 and 18 are strongly associated with cervical cancer; types 31, 33, 35, 39, 45, 51, 52, 56, and 58 are associated with a moderate elevated risk; and others, most commonly 6 and 11, are associated with genital warts, but not with cervical cancer [45].

The association of HPV infection and cervical cancer fulfils the criterion for causality proposed by Hill in 1965 [46]. In 1995, Walboomers *et al* [47] using archival negative cervical smears of 18 women who were later diagnosed with cervical cancer; concluded that HPV infection was present (up to six years) before development of disease. This has been confirmed by similar archival smears studies [48], and nested case-control or cohort studies using serology in blood samples [49,50,51,52,53]. Molecular studies have identified protein products of HPV early genes (E6, E7), which interact with growth-regulatory proteins of the human cell (p53, pRb), providing a possible mechanism for an HPV oncogenic effect [54,55]. Multiple epidemiologic studies have shown that the association of genital HPV with cervical cancer is strong (HPV infection was present in over 95% of cases) independent of other risk factors, and consistent in several countries [56,57,58,59,60,61,62,63,64,65,66]. Moreover, in 1999, Walboomers *et al* reported that the HPV prevalence in cervical carcinomas was of 99.7% in a worldwide series [3,67]. As a consequence, HPV is now widely recognised as the major causal factor of cervical cancer in the world.

HPV prevalence is age-dependent. Several studies have found a peak of infection before 25 years of age, and a decreasing prevalence afterwards [68,69,70,71,72,73,74]. However, a study by Cuzick *et al* [75] in women older than 35 years of age, showed that the prevalence of infection increases again in women older than 50. This pattern has been also observed in two more recent studies, with a clear second peak of HPV infection in women, with normal cytology (or ASCUS), aged 45 or older. Lazcano *et al* [76] in Mexico and Herrero *et al* [77] in Costa Rica; reported HPV prevalences in women

aged 18-25 of 17% or 20%, respectively, which rapidly decline into 4 to 5% in women 35-44 and start increasing again after 45 years of age. Possible explanations for this later infection peak include a cohort effect: longer exposure to HPV of these women when they were younger, or reactivation of latent HPV infections by reduction of immune surveillance or hormonal factors associated with older age.

Among sexually active asymptomatic women, 15% can have HPV infections, most of which are transient and will disappear spontaneously and are not important [78,79,80,35,81]. But recent evidence suggests that a subset of women will have persistent infections and therefore be at higher risk of developing cervical lesions. Median duration of persistence of infections varies between 8 to 14 months, depending on oncogenic types of HPV; 70% of infections will clear within one year, and less than 10% of women will continue to be infected after 2 years [82,83,84,85,86,87,88]. Factors determining persistence of HPV infection have not been established yet, though older age (>30 years), use of oral contraceptives (≥ 2 years), high viral load (RLU: relative light units>10) and presence of an oncogenic type in the first specimen have been suggested as potential ones in different studies [81,80,89,85,90].

Case series from many areas of the world have established that a high proportion (approximately 50%) of cervical cancers and high-grade CIN lesions contain HPV-16 DNA [2]. In a meta-analysis of longitudinal studies of unselected and low HPV prevalence populations, with follow-up for HSIL and cervical cancer in women tested for HPV antibodies, the authors estimated that vaccination against HPV 16 infection would prevent up to 44% of cervical carcinoma [91].

2.1.7.1. Other risk factors

Both descriptive and analytic studies have demonstrated that cervical cancer predominantly affects women in lower social classes, as defined by levels of income and education. Several studies have tried to established relationships between cervical cancer and age at first sexual intercourse,

lifetime number of sexual partners, sexual transmitted diseases (STDs), use of oral contraceptives, sexual behaviour of male partners, parity and smoking. Yet, to what extent, these are risk factors for cervical cancer, or are correlates of HPV infection, or are HPV cofactors operating only in the presence of the infection, or are independent risk factors is not entirely clear.

Recent studies have attempted to establish a role of Chlamydia Trachomatis infection as a cofactor of HPV on cervical carcinogenesis but still they have not shown consistent results [92], [93], [94], [95].

Latest reports from the IARC multicentric Cervical Cancer Study Group of a pooled-analysis of HPV DNA positive women of ten case-control studies (8 on invasive cervical cancer and 2 on in-situ carcinoma) done in Thailand [60], the Philippines [61], Morocco [62], Brazil [63], Peru [5], Paraguay [64], Colombia and Spain [65], [66], conclude that long-term use (5 years or more) of oral contraceptives [96] and high parity [97] increase the risk of cervical cancer, independently, after restricting analysis to HPV positive women. Women taking oral contraceptives for more than 5 years were 1.5 times more at risk than those not using them (Odds Ratio, OR: 4.01, CI: 2.01-8.02), and the risk doubles after 10 years of use (OR: 2.82, CI: 1.46-5.42 for 5-9 years, OR: 4.03, CI: 2.09-4.82 for 10 years or more), so the longer the use the higher the risk, and those who stop taking oral contraceptives at least 6 years before the study had risks almost similar to never users. These suggest that the risk and benefits from oral contraceptives, especially on women from countries with high incidence, needs to be re-evaluated. Treating the relative risk as a floating absolute risk to estimate CIs, nulliparous women were at less risk of squamous-cell carcinoma than parous ones, and the risk is increased with increasing number of full-term pregnancies; odds ratios of 3.8 (CI: 2.7-5.5) for women with seven or more full-term pregnancies compared with nulliparous ones and 2.3 (CI: 1.6-2.3) compared with women with one or two full-term pregnancies. No parity effect was found on risk of adeno- or adenosquamous carcinomas; odds ratio of 3.0 (CI: 0.6-14.6) for parous women compared with nulliparous.

Another report from the same group suggest that Herpes Simplex Virus Type 2 (HSV-2) may act in conjunction with HPV infection to modestly increase the risk of invasive cervical carcinoma and that this association is seen with both squamous-cell, and adeno- or adenosquamous carcinoma [98].

Smoking has also been independently associated with an increased risk of cervical cancer, a dose-dependent effect has been shown by different studies [99,100,101,102]. Nevertheless, some studies have reported no effect after adjusting for other HPV related life-style factors such as number of sexual partners and alcohol consumption [52,63,65,103].

More conclusive evidence comes from a systematic review (including 11 cohorts and 32 case-controls) of the association between cervical cancer and smoking by Szarewski and Cuzick [104], who reported statistical significant odds ratios for various groups of studies between 1.5 and 2.2. Moreover, both cotinine and nicotine have been found in cervical mucus of smokers [105,106,107,108]; and smoking cessation has been associated with reversal of CIN. In a longitudinal study [109] in which 81 women with CIN 1 or less were encouraged to quit smoking. After six months of follow-up, the lesions in 40% of those who stopped smoking disappeared, while it grew in those who did not stopped.

2.2. Prevention of cervical cancer

2.2.1. Screening

Screening consists of examination of asymptomatic people in order to classify them as likely or unlikely to have the disease for which they are screened. The disease could be cancer (as is when using mammography) or non-invasive neoplasia (as is the aim of cervical screening). Those who seem likely to have the disease are investigated further to arrive at a final diagnosis, and if considered diseased are supposed to be treated. The goal of screening is to reduce morbidity or mortality from the disease among screened people; this goal is accomplished by early treatment of identified cases [110].

Cervical cancer is a disease that can be prevented by detecting and treating its precursors. The Papanicolaou (Pap) test, which detects cervical dysplasia, is the primary method of screening for cervical cancer and its precursors. The aim of screening for cervical cancer is to identify and treat preinvasive lesions, thus preventing the progression to invasive cancer. This has been achieved in industrialized countries with organized mass screening programmes based on cytology [111,112,113,114,115,116,117,118,119,120]. However, substantial reduction in cervical cancer rates has been achieved only by numerous repeated tests over lifetime of women who undergo screening [121].

Age-adjusted incidence rates of cervical cancer (AAIR per 100,000 women) were reduced substantially in Denmark, Sweden and Finland, where the AAIR fell from 15 to 5 (65% reduction) from 1966 to 1985 after the implementation of well-organised mass screening [7,122]. The same has happened in the UK, where a 50% reduction in incidence rates during the last decade has been observed [123] and a possible similar reduction in mortality rates in women aged 20-69 years have been suggested [124] due to a well-organised screening programme with high coverage.

A collaborative study of screening programmes in eight countries: Scotland, Canada, Iceland, Denmark, Norway, Sweden, Switzerland and Italy; identified cancer cases (either from case-control or cohort studies) and selected previously screened healthy controls (or women in the cohort with an initial negative smear); and compared the relative protection acquired from cervical screening against developing cervical cancer. Women who had two or more negative smears were less likely to develop disease than those never screened, but the effect depended upon time from last negative smear. The relative "protection" was 15.3 for developing cervical cancer within one year of a negative screening in women with at least two negative screenings, and 1.6 within 10 years [113]. Sasieni and colleagues [115] also compared the screening history of women with invasive cancer to those of healthy controls (matched by age and residence) in England, Wales and Scotland, and confirmed that the risk of developing

invasive cervical cancer was a function of the number of years since a negative smear result. They found that the relative protection against cervical cancer was reduced from 5.6 to 1.6 within one year or 4-5 years after a negative screening. Other studies have confirmed that two or more previous negative smears have a protective effect on the development of cervical cancer, but that such effect decreases as time since the last negative smear increases, suggesting that although cytology based well-organised mass screening programmes have been very successful, they depend on a technique which has large false negative rates [125, 120, 126, 127]. As a consequence, women who are lesion-free at screening may experience the development of invasive cervical cancer within few years [48, 128, 129, 130, 131, 132].

These limitations have generated interest in the use of new alternative technologies such as liquid-based cytology and human papillomavirus (HPV) testing for more effective cervical cancer screening programmes; and other less-costly techniques for developing countries that so far, have failed to establish effective cervical screening programmes.

2.2.1.1. Cytology

The smear test (or Pap smear) consists of cells removed from the cervix; which are specially prepared for microscopic examination. A gynaecologist or other health care provider removes the cells by brushing or scraping the cervix during a pelvic examination. The removed cells are evenly spread on one or more glass slides. Pap smears are then stained, examined under a microscope, and interpreted.

As stated before, there is good evidence that smear tests can be used as part of an organised screening programme to effectively reduce the incidence of cervical cancer [113, 115]. However, the sensitivity is poor, and the accuracy of the test is subject to human error. Conventional cytology is limited by sampling error, in which the abnormal cells do not get placed on the slide, and reading error, whereby the few abnormal cells present are lost among the many normal cells that predominate. Sensitivities of 40% to 80% to

detect high-grade cervical intraepithelial neoplasia have been reported [133,134,71,135]. In particular, the relative sensitivity of cytology (LSIL or worse) for confirmed HSIL was reported to be 46% (CI: 35,57), on women attending family practitioner clinics for routine screening [71]. The second phase of the Zimbabwe study estimated a sensitivity of cytology of 44.3% (CI: 37,51) for HSIL [135]. These studies are consistent in finding that less than half of women with HSIL are identified by conventional cytology.

Furthermore, in a meta-analysis by Fahey *et al* [136], the mean sensitivities of cytology for detecting CIN 1 and CIN 2 were 63%(95%CI:55-71) and 61%(95%CI:52-70), and the specificities were 69% (95%CI:62-76) and 65%(95%CI:57-74), respectively. But a more recent meta-analysis by Nanda and collaborators [137], reported sensitivities for cytology at LSIL threshold for detecting CIN 1 from 18% to 98%, and for detecting CIN 2-3 from 23% to 99% in studies in which all women or a random sample with negative tests attended colposcopy. The correspondent specificities varied from 9% to 100% for CIN 1 and from 6% to 99% for CIN2-3, highlighting the variability of performance of conventional cytology.

Although established programmes in other parts of the world rely on cytology (Pap smears), recent research indicates that other technologies such as visual inspection after the application of acetic acid (VIA), HPV testing or liquid-based cytology (LBC) may be more efficient in detecting high-grade cervical lesions in developing countries [135,138,139,140,141,135].

Standards of cytology in Latin America are generally very poor [8]. The main problems being the high number of false negative and false positive results due to poor specimen quality, and high intra- and inter-observer variability in cytological diagnosis. Also problems regarding deficient infrastructure, insufficient and inadequate personnel training, shortage of human resources and clinical information, lack of supervision and follow up in procedures at laboratories, different diagnostic criteria, and classification systems, lack or delay in reporting, and lack of internal and external quality control. Diverse technical problems occurred in and out of laboratories, starting at the smear taking stage, where availability of

spatulas and slides may be scarce so slides are washed and reused. Papanicolaou smear providers do not take adequate samples, and slides get mixed up, lost or broken before they reach the laboratory.

The use of Liquid-based cytology (LBC) has been proposed as an alternative to conventional cytology in industrialised countries, and is being tested in less developed ones. LBC has been shown to be at least as sensitive as conventional cytology for detection of cervical lesions and is potentially more easily combined with (semi) automated reading of slides [142, 143].

LBC samples are collected using a plastic broom or a combination of plastic spatula and endocervical brush. Samplers are rinsed or the head of the brush is detached into a vial of liquid transport medium, creating a cell suspension. Once in the laboratory, slides are prepared either manually (labour-intensive) or in a semi- or fully-automated fashion using one of three devices approved by US Food and Drug Administration (FDA): two Thin-Prep® processors manufactured by Cytic Corporation and the SurePath® system previously known as AutoCyte-Prep® or CytoRich® manufactured by TriPath Imaging Inc.

Several split-sample or direct-to-vial studies have been carried to evaluate the performance of LBC either using ThinPrep or AutoCyte-Prep® systems; most of them showing consistent advantages of LBC over conventional cytology.

Liquid-based cytology reduces the number of inadequate samples and removes responsibility for slide preparation and fixation from the smear taker [144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154].

LBC slides are uniformly well fixed, free of inflammatory exudates and blood making them easier to screen [155, 156, 157, 158].

A systematic review of LBC by Payne *et al* [159], concluded that LBC might reduce the number of false negative test results, the number of unsatisfactory specimens and might decrease the time needed for specimen interpretation.

In the UK, debate about the implementation of LBC massively is on going; among opinions against the change are those of Herbert and Johnson [155], Moseley and Paget [160], and the latest results of a study by Coste and colleagues [161]. Herbert and Johnson re-evaluated the evidence in favour of LBC, and concluded that the cost of implementing LBC in a massive way would be overwhelming, especially because cytopathologists would need time to develop experience in reporting LBC and in assessing specimen adequacy; and LBC cytoscreeners would need constant training. In a more sophisticated review, Moseley and Paget [160] analysed published raw data using the National Health Service Cervical Screening Programme (NHSCSP) terminology to avoid differences due to nomenclature, especially regarding definition of inadequate samples. They also concluded that the benefits of LBC have not been sufficiently sustained to justify replacement of conventional cytology and more evidence in favour of LBC through multicentre trials is needed. A more recent study by Coste *et al* [161], after screened 828 women referred for colposcopy because of previously detected cytological abnormalities and 1757 women attending for routine smears, with conventional cytology, liquid-based cytology and HPV testing; conclude that LBC was more likely to give false positive and false negative results than conventional cytology.

After considering these opinions and deeply evaluating the results of Coste and colleagues, as well as previous literature and new studies, the reviewed NICE Guideline [154] on the use of liquid-based cytology for cervical screening concluded: that the sensitivity may be up to 12% better with LBC (to detect low-grade disease or worse) compared with Pap smear (conclusions based on a meta-analysis of 14 studies); that there is no difference between the specificity of LBC and Pap smear (based on a meta-analysis of six studies); that the rate of inadequate samples was reduced with LBC using data from a UK pilot study; which also gave evidence to support that the detection of glandular neoplasm with cytology is similar when using LBC or Pap smear. NICE recommended to use LBC as the primary means of processing samples in the cervical screening programme

in England and Wales, but that there is not enough evidence to recommend one LBC product over another. This guideline is to be reviewed in 2006.

Overall, LBC appears to be more sensitive than CC for detection of LSIL or worse lesions, and that the number of inadequate samples is reduced; but still is an expensive technique and requires lots of training.

2.2.1.2. Human Papilloma Virus Testing

High-risk types of HPV cause at least 95% of cervical cancer [162,3], but the majority of women exposed to genital HPV will not develop cervical cancer [45].

It has been proposed that HPV testing can be used as an adjunctive to cytology or even as a primary screening test. HPV testing has poor specificity but it has higher sensitivity than cytology for detecting HSIL on histology. Both HPV testing and LBC reduce the inadequate rates, because of the collection devices and the transport media used and the reduced involvement of the smear takers. HPV testing of self-collected samples still has better sensitivity than cytology and could potentially be used for women who do not wish to attend screening in developed countries [163,164, 165].

The use of HPV testing in the triage of ASCUS and low-grade disease and in the follow-up of treated women is under study in pilot centres in the UK. If HPV proves being efficient in either of these areas as has been suggested for ASCUS by two very large US studies [166,167,168], clinical management of minor lesions and of treated women could be substantially improved.

Several techniques have been used to detect HPV in cervical smears giving different results. In a systematic review of the role of HPV testing within a screening programme, Cuzick *et al* established that two consensus primer PCR systems (MY09/11 and GP5+/6+ pairs) and Hybrid Capture II (HC-II) using high-risk probes are the best methods so far because they have high sensitivity for detecting oncogenic viruses and could be automated [169].

Most studies using HC-II have reported sensitivities over 80% and specificities between 62% and 98% to detect histologically confirmed high-grade lesions or worse [75,170,171,172,173,174,161] confirming that HC-II is a sensitive and robust test for oncogenic HPV that can be used to identify women with high-grade cervical lesions in a screening setting [169].

The test is currently expensive for a developing country and may also lack specificity. However, unlike earlier PCR based techniques, HC-II is relatively straightforward to implement in hospital laboratories. Aliquots of cells stored in a liquid-based medium may be analysed either by thin layer cytology or by Hybrid Capture for HPV DNA, creating the opportunity to triage the management of patients with cervical abnormalities.

2.2.1.3. Visual inspection after acetic acid application – a systematic review

The naked-eye visualisation of the cervix after application of 3-5% acetic acid is termed cervicoscopy [175] or visual inspection of the cervix after acetic acid (VIA). The results are reported as negative (no acetowhite areas) and positive (evidence of acetowhite) areas, but other more detailed descriptions are possible.

VIA has been investigated as a low-cost alternative to cytological screening programmes in countries where skilled labour costs are low. VIA with or without magnification has shown very similar or better sensitivity to cytology in detecting pre-cancerous lesions. It does not require a cytological laboratory, it is cheaper than cytology and the outcome is immediately available.

After searching MEDLINE and the "Web of Science" electronic databases, all papers with "visual inspection", "VIA", "DVI", "acetic acid test", "AAT" "cervicoscopy" and "visual methods" in their title, abstract or keywords were selected and reviewed. Studies with colposcopy (and histology) used as reference standard to estimate sensitivity and specificity of VIA for detecting intraepithelial lesions are presented in Table 2.2.1.3.1. As sensitivity and specificity are often biased by the design of the studies, the percentage of VIA positive tests in women with histologically

confirmed HSIL or worse lesions (% VIA pos in HSIL pos) and in those with lesions no worse than LSIL are presented (% VIA pos in <HSIL) instead. The last two columns show the number of women who attended colposcopy, the corresponding percentage from all women in the study, and the criteria used for referral to colposcopy.

The first report indicating that a cervix at risk can be identified by recognising acetowhite areas with the naked eye; was that of Ottaviano and La Torre [176]. The cervices of 2,400 unselected patients with normal or abnormal cervical cytology were examined in Florence, Italy, by both VIA and colposcopy; and their results were compared. VIA and colposcopy were considered positive if the transformation zone was found atypical (ATZ). Punch biopsies were taken during examination if appropriate. Histology results were reported in four categories: benign lesions, cervical intraepithelial neoplasia (CIN) grades I and II, CIN III and preclinical invasive carcinoma. An ATZ was found in 312 women with colposcopy. VIA gave the same result for 307 of these women but classified as "suspicious" 5 of them. Histologic examination of biopsies from the 312 ATZ revealed 169 (54.2%) benign lesions, 81 CIN I-II, 56 CIN III and 6 preclinical invasive carcinomas. The five cases classified as "suspicious" by VIA were histologic benign lesions. Based on the high percentage of agreement between colposcopy and VIA, the authors concluded that colposcopic magnification was not essential in clinical practice to identify the cervix at risk, but was useful to decide how to treat cases of CIN.

As this study intended to highlight the importance of the naked-eye inspection of the cervix by gynaecologists, results from Pap smears were not presented nor was their correlation with VIA or colposcopy commented upon.

Following Ottaviano *et al* conclusion, that the naked-eye visualisation of the cervix was enough to detect a cervix at risk, Ficsor *et al* [177] carried out a study in rural clinics of Van Buren, Michigan, USA, to determine if additional women at risk for cervical cancer were identified when using VIA, ViraPap and ViraType tests. A Dacron swab and a wooden spatula

were used to collect endocervical and cervical cells that were then spread into the same glass slide for conventional cytology evaluation. Cells remaining on the wooden spatula were collected on the Dacron swab, and then immersed in a transport media provided with the ViraPap kit, which was also used to test HPV DNA. Those found positive to HPV DNA were then analysed using the type-specific ViraType DNA test kits. They found that 30 of 145 (21%) women were VIA positive (abnormal acetowhitening of the cervix) as compared to 13 HPV DNA positive and 14 (10%) who had some cervical abnormality detected by conventional cytology. Twenty-two women were positive on VIA but had a negative Pap smear and a negative ViraPap test. In 6 of 14 (43%) patients with abnormal Papanicolaou tests no acetowhite lesions were detected. Positive Pap test results were 6.61 times more likely when VIA was positive and 5.41 times more likely when HPV DNA was detected in cervical cells. Based on these results and on the fact that the Pap test is considered an effective screen for early cervical cancer and its precursors, the authors suggested that VIA and the ViraPap test also detect women who are at risk for cervical cancer. Unfortunately, no definition of positivity on each test was stated clearly and since colposcopy was not performed, and hence histology was not available, true disease was not determined and sensitivity and specificity were not estimated. These results are not included on Table 2.2.1.3.1.

Conventional cytology and VIA were used to screen 2827 women less than 46 years of age in the HARNET study area in Pennsylvania, USA, by Slawson and associates [178]. Cytology was considered positive if ASCUS/AGUS or worse lesions were found. VIA was considered positive if acetowhite areas were detected "outside the transformation zone (sic)". Women with Papanicolaou smears showing squamous intraepithelial lesions underwent immediate colposcopy, while those with a positive VIA (cytology less than SIL) had colposcopy within a 6-month period. Specially qualified doctors performed colposcopy and directed biopsies. Endocervical curettage was performed on all subjects. Of the 2827 women screened, 358 (13%) were found to have an abnormal result on VIA or cytology, 74 of these did not meet the inclusion criteria (47 older than 45

years of age, 20 had previous cryotherapy, 7 were pregnant); and 63 eligible women refused colposcopy. VIA correctly identified 43, found 14 additional CIN I lesions and 4 additional CIN II or III lesions that were missed by cytology, representing a 27.3% increase in the detection of cervical lesions. However, in almost 50% of positive VIA cases, colposcopic findings were normal.

In the study of Van Le *et al* [179], 85 women positive on VIA but with normal cytology, were subjected to colposcopy. Of them, 34 (40%) had normal colposcopic examinations, and the rest were subjected to biopsy. Thirteen (15%) CIN lesions (nine CIN I and four CIN II) were detected by VIA and missed by cytology, but 34 false-positive women underwent unnecessary colposcopy. Because women with VIA negative, were not referred for colposcopy a false-negative rate could not be estimated.

Cecchini *et al* in Florence, Italy [180] screened 2105 women using VIA, Cervicography (projected magnified inspection of pictures of acetic acid impregnated cervix), and cytology. VIA was reported positive if there was evidence of acetowhite areas. Cytology was considered abnormal if ASCUS/AGUS or more severe lesions were reported. Any woman with abnormal cytology, suspicious Cervicography or positive VIA was invited for colposcopy. Negative colposcopy or histologic diagnosis at colposcopically guided biopsy, were considered as the gold standard for the determination of sensitivity, specificity and positive predictive value for CIN II-III (HPV morphology or CIN I were considered negative). Among 486 women with at least one positive test who underwent colposcopy, directed biopsies were performed in 281. VIA, cervicography, and cytology detected 7, 5, and 5 of the 8 high-grade lesions (4 CIN II and 4 CIN III) histologically confirmed. VIA was found to be more sensitive than cytology in detecting lesions, but because of being less specific resulted in a recall of 25% of 2,105 subjects for further investigations, as opposed to 4% with cytology. Because colposcopy was performed only in 23% and biopsies were taken only on 13% of screened women, the definition of specificity was limited.

Frisch et al [181], reported a significantly augment on the ability of screening to identify normal women when adding VIA to cytologic screening in a selected population of college students with a high prevalence of low-grade squamous lesions. The negative predictive value for detecting HPV infection or cervical intraepithelial neoplasia (CIN) increased from 67%[CI: 57-77] for cytology alone to 91%[CI: 83-99] when performing VIA as an adjunct. Unfortunately, this study did not have enough power to evaluate the performance of VIA in detecting HSIL.

Another study by Slawson and associates [182] on the HARNET population evaluated the use of VIA in the follow-up of Papanicolaou smears reporting ASCUS. They found that VIA increased the detection rate of CIN II or worse lesions from 67% to 93% and proposed a management scheme to minimise unnecessary colposcopy by performing it if either repeated VIA or cytology after 4 or 6 months was positive. This study is not included in Table 2.2.3.1.1, because women participating in their first study [178] are included to women recruited the next 10 months.

In a study in Cape Town, South Africa [183], the positive predictive value for detecting histologically confirmed LSIL of VIA was 72.4% as compared with 89% of conventional cytology. Women, attending a mass free screening program at a mobile clinic, were screened by VIA and conventional cytology. Smears were processed immediately by a cytotechnologist aware of the study. Those being positive on either test underwent colposcopy and were treated with large loop excision of the transformation zone (LEEP) when necessary within three days of being screened. Unfortunately, those resulted negative on both tests were not further evaluated and so estimates of sensitivity and specificity were not possible to obtain. The authors concluded that the higher number of false negative tests with VIA than with cytology made VIA not useful as a screening method for premalignant cervical lesions. But since VIA detected 64% of HSIL on both cytology and histology, they suggested that it should be considered as a possible alternative to cytology in countries with limited resources or wherever cytology is not available.

Five hundred sexually active, non-pregnant women attending a college hospital in Vellore, India [184], were screened with VIA and conventional cytology (sample collected immediately after VIA), and were asked to return for colposcopy. Of the 500, 372 underwent colposcopy, all information presented in the paper is based in this colposcopically examined women. Biopsies were collected during colposcopy if indicated and colposcopy diagnosis was used as the reference test (gold standard). VIA was positive in 197 women (53%) while only 23 women (6.2%) were positive on conventional cytology (disease threshold not specified). Seventy-five women were colposcopically diagnosed with LSIL, 22 with HSIL and one had a histologically confirmed invasive cancer. The respective sensitivities were 72.4% and 13.2% for VIA and conventional cytology, and the correspondent specificities were 54% and 96.3%. Using colposcopy as the standard test, VIA missed 5 HSIL while cytology failed to detect 17 HSIL and one invasive lesion, but the false positive rate of VIA was 91% to detect high-grade lesions, implying a large number of unnecessary colposcopies. Still based in the gain in sensitivity the authors concluded that VIA was a promising screening test for developing countries and that the high false positive rate could be decreased with adequate training of VIA examiners. It is noteworthy that smear samples in this study were taken after application of acetic acid and others have shown that this can adversely affect the results [185].

Sankaranarayanan et al [138], concluded that VIA and cytology had similar performance in detecting cervical lesions in a study in Kerala, India. Considering a low-threshold for cytology (defined positive if atypia or a worse lesion was present), detection rates were 15.7 and 15 per 1000, the specificities were 92.2 and 92.7 and positive predictive values were 17 and 17.2 for VIA and cytology, respectively, to detect histologically confirmed HSIL or worse lesions. But negative predictive values were not possible to obtain, again, because only those testing positive and those with a macroscopically abnormal-looking cervix underwent colposcopy. However, the high specificity obtained for VIA as compared with previous studies was explained by prolonged training of those performing the exam and by the definition of VIA results, which were considered positive only if distinct

acetowhite areas were present. In a selected population of 1,351 women from this Kerala study [186], the authors reported detection rates of HSIL and cancer of 53.6 and 34.7 per 1000 for VIA and cytology, and approximated specificities of 68% and 89.5%, respectively. Because these women were attending a cancer detection clinic, either for a routine exam or as referred to rule out cancer, VIA showed a significantly higher detection rate (p-value <0.01 for a ratio of sensitivities of 1.54) than cytology. But VIA specificity was lower than that of cytology (p-value<0.01) yielding a higher referral of women for colposcopy. This second study is not summarised in Table 2.2.3.1.1 since study populations overlapped.

In a pilot study in Zimbabwe, Chirenje et al [187] screened 1000 women aged 25-55 years coming into primary health clinics in Mashonaland with conventional cytology and VIA. Colposcopy was performed on any women with an abnormal result in either screening test and in a 10% random sample of those negative in both VIA and cytology. Using colposcopy as the gold standard, the sensitivity of VIA was 68% and the specificity 3.4%, despite this extremely low specificity the authors concluded that VIA was a practical alternative to cervical cytology in countries with limited resources.

The second-phase study in Zimbabwe [135] tried to overcome the lack of the false-negative rate. In the first phase, women testing positive on either VIA or cytology, as well as a random sample of those negatives on both tests were scheduled for colposcopy. In the second phase of the study all women regardless of results were called for colposcopy.

A total of 8,731 women were enrolled on phase I, of them 1,584 had colposcopy and biopsy as indicated. Positivity rates for VIA and cytology were 20.2% and 14.6%, and the predictive positive values were 25.9% and 43.9% for detecting colposcopically or biopsied confirmed HSIL or worse (assumed as true disease), for VIA and cytology, respectively.

During the second phase of the study, 2,203 women participated and 2,147 of them underwent colposcopy. Also, an additional sample for HPV testing was collected to determine if feasible as a single screening test in a less-developed

country and to assess its use as an adjunct to VIA. Sensitivity and specificity of VIA were 76.7% and 64.1%, as compared to 44.3% and 90.6% of cytology, to detect HSIL or worse. The authors concluded that their results confirmed once more that VIA is more sensitive but less specific than cytology, but still VIA could be used as a screening test in difficult settings even though the expected large number of referrals. However, the different rate of high-grade disease found in both phases is of concern (4.7% in Phase I and 9.5% in Phase II), since the sensitivity of VIA was only increased in 10%.

Denny and colleagues carried out two studies in South Africa: one in Cape Town (2944 participants) between January 1996 and September 1997 [139] and a second one in the periurban area of Cape Town (2754 participants) between January 1998 and November 1999 [188]. In both studies women were screened with four tests: VIA, conventional cytology, HPV testing and Cervicography. As in the Zimbabwe study, the rate of high-grade disease increases from 2.9% to 4.2%, while the sensitivity of VIA only increases 5%. In both studies a magnified visual inspection was performed immediately after VIA, with the intention to increase the sensitivity of VIA, but made no significant improvement. The specificity of VIA was 83% in the first study and 78% in the second one. Once again, the authors conclude that VIA could be used as a primary screening test in low-resource settings.

In another study in New Delhi, India [189], 402 symptomatic women attending a gynaecological clinic were examined with VIA, conventional cytology and underwent colposcopy. The sensitivity of VIA for HSIL was 87% and the specificity 82%, but as expected this population had an extremely high rate of HSIL (34%).

Table 2.2.1.3.1. Summary of screening studies evaluating Visual Inspection after acetic acid (VIA).

Authors	No. screened	% with HSIL	% VIA pos in HSIL pos	% VIA pos in LSIL pos	% VIA pos in <LSIL	% VIA pos in <HSIL	No. with Colp. (%)[a]	Criteria used for referral to colposcopy
Ottaviano M *et al*[1] Italy 1982	2400	2.6	100			10.5	2400 (100)	All women.
Slawson DC *et al* USA 1992	2753	1.1	29	37.7	2.2	2.9	221 (8)	VIA+ or cytology ≥ASCUS.
Van Le L *et al*[2] USA 1993	85	4.7	100	36.5	58.8	95.3	85 (100)	All women. But women VIA negative not described in the study.
Cecchini S *et al* Italy 1993	2105	0.4	87.5			16.5	486 (23)	VIA+, cytology ≥ASCUS, or cervicophotography.
Frisch LE *et al* USA 1994	95	4.2	100	92.3	70.5	73.6	52 (55)	VIA+, cytology ≥ASCUS, or cervicophotography.
Megevand E *et al* South Africa 1996	2426	1.3	64.5	13.8	1.0	2.3	330 (14)	VIA+ or cytology ≥LSIL.
Londhe M *et al* India 1997	372*	6.2	78.3	70.7	46.0	51.3	372 (100)	All women. Colposcopy results were used as reference test*.
Sankaranarayanan R *et al,* India 1998	3000	1.7	90.2	70.1	6.1	7.8	277 (9)	VIA+ or cytology ≥ASCUS, and those with abnormalities on speculum exam.

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Table 2.2.1.3.1. Summary of screening studies evaluating Visual Inspection after acetic acid (VIA).

Authors	No. screened	% with HSIL	% VIA pos in HSIL pos	% VIA pos in LSIL pos	% VIA pos in <LSIL	% VIA pos in <HSIL	No. with Colp. (%)[a]	Criteria used for referral to colposcopy
Chirenje ZM *et al* Zimbabwe 1999	1000	3.8	68.4			23.3	213 (21)	VIA+ or cytology≥HSIL. Colposcopy was the reference test.
JHPIEGO Zimbabwe 1999	8731 Phase I	4.7	65.5			17.9	1584 (18)	VIA+ or cytology≥LSIL, and a 10% of negative or atypical VIA women.
JHPIEGO Zimbabwe 1999	2203 Phase II	9.5	76.7	54.1	32.7	35.9	2147 (98)	All women.
Denny L *et al* South Africa 2000	2944	2.9	67.4	49.5	18.3	16.7	760 (26)	VIA+, cytology≥LSIL, HPV (viral load>10pg/mL), or cervigrams: "warranting colposcopy" or ≥LSIL.
Singh V *et al* India 2001	402	33.6	87.4	52.1	6.0	18.4	402 (100)	All women. But selected women with gynaecological symptoms.
Belinson JL *et al* China 2001	1997	4.3	70.9	44.1	24.4	25.7	1997 (100)	All women.
Cronje HS *et al* South Africa 2001	6301	2.6		49.4	16.0		1747 (28)	VIA+, cytology≥LSIL, or cervigrams: "warranting colposcopy" or ≥LSIL.

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Table 2.2.1.3.1. Summary of screening studies evaluating Visual Inspection after acetic acid (VIA).

Authors	No. screened	% with HSIL	% VIA pos in HSIL pos	% VIA pos in LSIL pos	% VIA pos in <LSIL	% VIA pos in <HSIL	No. with Colp. (%)[a]	Criteria used for referral to colposcopy
Denny *et al* South Africa 2002	2754 Unaided	4.2	72.6	57.8	20.7	22.2	1156 (42)	VIA+, cytology ≥LSIL, HPV (viral load>1pg/mL), or cervigrams: "warranting colposcopy" or ≥LSIL.
	Magnified		76.1	59.8	23.4	24.8		
Rodriguez-Reyes E *et al,* Mexico 2002	376	13.6	92.2	100	40.3	41.2	376 (100)	All women.
Claeys *et al* Nicaragua 2003	1076	4.7	88.2	84.4	22.4	27.0	307 (29)	VIA+ or cytology ≥ASCUS.
Sankaranarayanan R *et al,* India 2003	4444 low-threshold	3.3	88.6			22.0	4444 (100)	All women.
	high-threshold		82.6			13.5		

HSIL=HSIL or worse lesions.

a : percentage of women who underwent colposcopy in the study.

1: HSIL not including CIN II or moderate dysplasia.

2: HSIL including only CIN II. LSIL including koilocytosis.

* Originally 500 women, no data on 128 assumed without colposcopy.

A comparative trial of multiple cervical screening techniques in China [172] examined 1997 women aged 35-45 with HPV testing, fluorescence spectroscopy, liquid based cytology, VIA and Colposcopy. All women had multiple cervical punch biopsies. The sensitivity and specificity of VIA were 71% and 74%, respectively. These estimates were free of verification bias as each woman had histology. These results were not as good as those from Zimbabwe or India. Furthermore, VIA was performed by gynaecologic oncologists, who missed one third of the cancers identified during the study.

Cronje *et al* [190] screened 6301 women with conventional cytology, cervicography and VIA in the Free State Province of South Africa. They reported a sensitivity of 49% and a specificity of 84% of VIA for detecting CIN I or worse lesions. VIA results by histology are not presented limiting the calculation of measures of performance to detect HSIL.

The first study published in Latin America has been that of Rodriguez-Reyes and colleagues in Mexico [191]. They examined 376 women with VIA and colposcopy (and directed cervical biopsy) attending an early cancer detection program in Durango, Mexico. Because of being a selected population the rate of high-grade disease was high (14%), nevertheless, VIA had a sensitivity of 92% but a low specificity of 60%.

A recent study in Nicaragua [192] screened 1076 women with VIA and conventional cytology. VIA was performed by six medical doctors and 14 trained nurses. Women testing positive in either test were referred to colposcopy and had biopsy if indicated. VIA had a sensitivity of 88% and a specificity of 73% but only 29% of the participants underwent colposcopy. The authors concluded that VIA increases the detection of HSIL significantly as compared with conventional cytology (positive if ASCUS or worse); which had a sensitivity of 45%; and that uniform criteria on test positivity should be established in order to improve VIA performance in field conditions.

Another recent study in Kerala, India [193] evaluated 4444 women with conventional cytology, VIA and visual inspection after the application of Lugol's iodine (VILI). VIA test positivity had a low and a high threshold

definition. The later including well-defined opaque acetowhite lesions close to or touching the squamocolumnar junction. They reported a rate of high-grade disease of 3%, sensitivities of 89% and 83% and specificities of 78% and 87% for low and high VIA thresholds, respectively. This results are unbiased as every woman underwent colposcopy and are more realistic than those of the previous study in 1998 where sensitivity and specificity of VIA were over 90%.

It should be stated that only three studies were free of selection and verification bias: the second phase of the JHPIEGO study in Zimbabwe, the study by Belinson and colleagues in China, and the recent one of Sankaranarayanan in India. The sensitivity of VIA could then be assumed to vary between 70% and 89%, and the specificity between 64% and 78% for detecting histologically confirmed high-grade lesions.

Soler *et al* [194], in a review of the new technologies for the detection of cancer precursors, stated that despite the higher rates for referral or an offer to treatment obtained with VIA, the gain with increasing coverage, diminishing losses to follow-up and immediate results, are to be considered. They suggested that implementing two-step approaches using adjunctive HPV self-sampling or sequential screening with HPV or cytology may improve the specificity of VIA alone.

There are several studies on going in order to evaluate the efficacy of combined cervical screening tests in India, South Africa and in Peru, results of which are to be available in the following years. However, so far, despite its poor specificity, VIA has been shown to be a potential alternative for cervical screening in developing countries.

2.2.2. Vaccination

The objective of vaccination is to prevent cervical cancer, by immunizing against HPV infection or eliminating persistent infections (prophylactic vaccines) or treating women already infected with HPV (therapeutic vaccines).

In 1991, Zhou and colleagues created the first papillomavirus-like particles in the laboratory [195]. Virus-like particles are non infectious as they contain no DNA or RNA, but they imitate the natural structure of the virion and generate a potent immune response. Vaccines derived from species-specific virus-like particles protected animals from wart viruses. The next step was then to determine if such vaccines will indeed protect cervical epithelium from high-risk HPV infections [196]. A recent study by Koutsky and collaborators [197] evaluated the protective effect of a virus-like particle vaccine against HPV 16. A total of 2,392 American women aged 16-23 years were randomised to receive three doses of placebo or HPV-16 virus-like-particle vaccine intravenously (40 μg per dose). In a first analysis of 765 placebo women and 768 vaccinated women followed-up for a median of 17.4 months, they reported an incidence of persistent HPV-16 infection of 3.8 per 100 woman-years at risk in the placebo group and zero in the vaccine group; and the only nine cases of HPV-16-related cervical intraepithelial neoplasia were all in women receiving placebo. The authors conclude that this prophylactic vaccine against HPV-16 infection is highly efficacious, but long-term follow up studies are needed to determine the actual duration of its protective effect. Also, "multivalent vaccines", those that can prevent more than one HPV type, are already under study and could prevent more cancer cases. Therapeutic vaccines are at a much earlier stage of development [198].

Although, vaccination is indeed a promising alternative to cervical screening, the availability and introduction of them in a massive scale in developing countries could take many years, and cost-effective analysis are needed before deciding large interventions.

2.3. Peru

Peru is located in west central South America, bounded on the north by Ecuador and Colombia, on the east by Brazil and Bolivia, on the south by Chile, and on the west by the Pacific Ocean. The area of the country,

including several offshore islands, is 1,285,216 sq. km, making it third in size (after Brazil and Argentina) of South American countries.

Peru may be divided into three main topographical regions: the coastal plain, the sierra (highlands) and the Amazon forest. Its climate varies widely, ranging from tropical in the Amazon forest to arctic in the highest mountains of the Andes.

The population of Peru is estimated to be 25,661,700 with 50.4% females, 33.4% children (under 15 years of age) and only 4.8% people over 64 years (estimates based on the National Census of 1993[3]. About 45% of Peru's inhabitants are Indians, some of whom are descended from the Inca civilisation of the 15th century. Some 37% of Peruvians are mestizos, mixed white (mainly Spanish) and Indian background, 15% are of unmixed white descent and many of the remainder are of black African extraction, or Asian (Japanese, Chinese and Korean).

The overall population density of Peru is about 20 persons per sq. km. The distribution, however, is uneven, the coast is only ten percent of the territory but 40% of Peruvians inhabit it, the Sierra (31.8% of Peruvian land) holds 37% of Peruvians, and only 10% live in the Amazon forest, which is the largest region (739,672 sq. km, 58% of Peruvian territory). Around 72% of Peruvians live in urban areas, with 29% living in Metropolitan Lima, 22% in other major cities, and 21% in other urban areas.

Metropolitan Lima is the capital city of the country. It is situated in the central part of the Pacific coast. It is by far the most populated city, with 7,497,000 inhabitants follow by Arequipa with 762,000 inhabitants, Trujillo (652,000), Chiclayo (517,000) and Iquitos (367,000). During the last five decades, there have been internal migrations, from deprived parts of Peru to more developed areas, mainly to coastal cities such as Lima, which has been the favourite target. Most people migrate from rural areas, expecting better opportunities and life improvement (housing, employment and education); instead, they usually experience a lot of deprivation while adapting. Accommodation and education are expensive, the unemployment

and sub-employment rates are very high, and social discrimination is a huge problem.

Spanish, the only official language until 1975, is spoken by 70% of the population. Quechua, one of the main languages of the Incas, was made official in 1975. Aymará, another Incas language, is also spoken in some parts of the country.

Peru is a catholic country, more than 90% of Peruvians profess this religion. In 1915, Roman Catholicism was established, as the official religion of the country, however few aborigens still practice customs of their inherited religions. There are also a small number of Jews and Muslims, and a larger number are becoming Protestants.

The estimated Peruvian birth and mortality rates are of 23.7 and 6.3 per 1000, respectively. The average annual growth rate is 1.7% and life expectancy is 69.1 years.

2.3.1. Health system in Peru

The health system of Peru is composed of public, social security, army forces and private facilities, all of them under the regulation and monitoring of the Ministry of Health, the highest authority.

The country is divided into health regions composed of health networks that include hospitals, clinics, health centres and health posts. There are more than 7,800 health facilities all over the country. Most of them are concentrated in Lima and other urban areas, despite an effort for decentralization that started in the nineties. Primary care is not free except in some deprived areas of the country where family planning and reproductive health clinics, as well as children vaccination are covered by the health region budget. But in most places, patients are to pay their cost for hospitalisation and basic medication.

Only those with a permanent job are covered by the social security system, and those enrolled or with family working in the army forces can access the army forces facilities.

Private hospitals and clinics are expensive and so only available to a small sector of the population.

2.3.2. Cervical cancer in Peru

Cervical cancer is the most common cancer in women in Peru, as shown by figures from the only two existing cancer registries in the country the Trujillo Cancer Registry (TCR) and the Metropolitan Lima Cancer Registry (MLCR).

In 1994, 880 new cases of invasive cancer were diagnosed at, the Instituto de Enfermedades Neoplasicas "Dr. Eduardo Caceres" (INEN), the national cancer hospital in Lima. Most of these cancers were on late stages with no possibility of therapeutic treatment.

In 1998, the Ministry of Health of Peru (MOH) formulated a National Plan for Gynaecological Cancer Prevention: Cervix and Breast 1998-2000, in which activities to be carried out at each health level to implement a national screening programme, were detailed.

In 1999, once the plan was revised and approved, cervical cancer was declared a health priority in the country.

2.3.2.1. Mortality and incidence, rates and trends, importance in Peru

The incidence rates of cervical cancer in Peru are high. In Volume VII of Cancer Incidence in Five Countries [199], the TCR reported 53.5 new cases per 100,000 women, the second highest rate in Latin America after that of Belem, Brazil (64.8 new cases per 100,000).

Trujillo is located in the La Libertad region, and its cancer registry covers five districts in the province of Trujillo with approximately 613,000 inhabitants, representing 2.3% of the Peruvian population [200]. Data from Lima are available from the Metropolitan Lima Cancer Registry, which covers the provinces of Lima and Callao with more than 8 million inhabitants, 32% of the Peruvian population [201].

In Metropolitan Lima, the annual age-standardised incidence rates of cervical cancer per 100,000 women were 45 between 1968-1970 and 27.3 between 1990-1991 while the crude mortality rates were 12.7 and 9.3 per 100,000 women in the same periods. Both, incidence and mortality have experienced a substantial decrease over 25 years in Lima, where cytology screening has been available since 1953, in a small scale from both public and private health services.

This has not happened in Trujillo, where rates have experienced a very small (non-significant) decrease over a decade (1984 to 1995), the incidence rate was 56.6 per 100,000 in 1984 and still there are more than 50 new cases per 100,000 women each year. Mortality rates were 20 per 100,000 women in Trujillo in 1984 and remained the same in 1995.

Unpublished data from the only specialised cancer hospital of the country, the Instituto de Enfermedades Neoplasicas "Dr. Eduardo Caceres G." (INEN) based in Lima, reported a total of 880 new cases of invasive cervical cancer, 139 in situ carcinomas and 111 cervical dysplasias admitted in 1994. Most of the invasive cases presented at late stages with no possibility of therapeutic treatment. Sixty percent of those occurred in women from other parts of the country. It is expected then that a larger number of cases are presenting all over the country and only those who can afford it would reach the cancer hospital.

Apart from INEN, there exist other cancer treatment units, but radiotherapy is available only in Trujillo and Arequipa, where the number of cases of cervical cancer is again large, and most cancers are diagnosed in very late not curable stages.

There is no doubt then that cervical cancer is not only the most frequent cancer in Peruvian women but also the major cause of cancer death.

2.3.2.2. Possible reasons for the high rates

Cervical cancer usually strikes poor non-educated women. That is the case in Peru where most cervical cancers occurs in women living in very deprived semi-urban and rural areas. Age is also to be considered, cervical

cancer incidence increases with age, and women between 35 and 50 years are at high risk, 35% of women in Peru are in this age group.

Since some high-risk HPV types are necessary to cause cervical cancer, although affected by age, the prevalence of HPV DNA infection is to be accounted. Unfortunately only two studies in Lima (one unpublished) have reported data regarding this. The first one carried out in a deprived area in Lima between 1993-1995, reported a prevalence of 20% of HPV DNA infection detected by PCR (unpublished data). The second one, a hospital-based case-control study in INEN, reported a prevalence of 17.7% of HPV infection among 175 hospital controls [5]. Both prevalences are within the range of similar studies. However, the prevalence of HPV 52 in cancer cases was 8.1% (14 of 173 squamous cell carcinomas), suggesting that HPV 52, now consider a high-risk type [202], is particularly confined to Peruvian women.

There exist national statistics on potential cofactors such as age of first intercourse, estimated to be 19.3 years, use of contraception methods, and other sexual behaviour data. None of them seem to explain the high incidence of cervical cancer, nor does smoking, since very few women smoke in Peru.

Because cervical cancer is a preventable disease, one reason for having high incidence and mortality rates in Peru is the lack of a prevention programme of cancer of the cervix uteri.

2.3.3. Efforts to control cervical cancer in Peru

Tables 2.3.3.1 and 2.3.3.2 show unpublished data presented by the Ministry of Health personnel in a working meeting in 1997. They reported a total of 401,155 cervical smears among 8,087,875 Peruvian women older than 14 years of age (5% coverage), 75% of those were screened for the first time. Forty one percent of those smears (163,381) were taken on non-pregnant women between 30 and 49 years of age.

Table 2.3.3.1. Number of Peruvian women screened with cytology in 1997.

	Number of screened women by age-group		
	15-29	30-49	≥50
Previously unscreened	174,952	113,612	13,918
Previously screened	46,541	49,769	5,729
Total number screened	221,493	163,381	19,647
Women to be covered *	3,550,394	2,862,847	1,674,634
Coverage	6.2%	5.7%	1.2%

* Projected female population for 1997 according to the 1993 National Census.

The cytology positivity rate was of 8% for mild dysplasia or worse. A total of 8,713 (27%) low-grade lesions, 6,138 (19%) moderate dysplasias, 5,383 (16%) severe dysplasias, 5,534 (17%) in situ carcinomas and 6,833 (21%) invasive cancers were cytological detected. Forty eight percent of high grade lesions were on young women (15 to 29 years of age), 31% in those between 30 and 49 years and 41% in those older than 49. Despite these large figures, information regarding treatment and follow up of these cases is not available.

Table 2.3.3.2. Cytology results of Peruvian women in 1997.

Cervical lesions detected by Conventional Cytology	Age-group		
	15-29	30-49	≥ 50
Low-grade lesions	3,988	3,942	781
High-grade lesions or worse	11,380	7,277	9,913
- Moderate Dysplasia	2,999	2,636	502
- Severe Dysplasia	2,777	2,185	421
- In situ carcinoma	2,759	2,232	540
- Invasive cancer	**2,845**	**2,860**	**1,127**
Total detected lesions	15,368	13,855	3,371

Albujar reported that in 1993, the region cytology screening coverage in women aged 15-49 years was 9.4%, 16.3% for the province of Trujillo, and zero for the highland and Amazonian areas of La Libertad region [203]. According to unpublished local statistics, the annual cytology coverage for 2000 was of 16% of women aged 15-49 in La Libertad, of them, only 39% were previously unscreened.

Jeronimo *et al* [204] evaluated Pap smear results of 61,846 screened in the gynaecological department of a general hospital in Lima, Peru, between 1994 and 1996, and found a positivity rate (for LSIL or worse) of just 0.47%. Given the very high rates of cervical cancer in Lima, these data suggest a very low quality of cytology. By comparison, approximately 3.5% of women screened in England have LSIL or worse on cytology.

2.3.3.1. General governmental efforts

In 1998, the Ministry of Health of Peru formulated a National Plan for Gynaecological Cancer Prevention: Cervix and Breast 1998-2000, in which activities to be carried out at each health level to implement a national screening programme, were detailed.

In 1999, once the plan was revised and approved, cervical cancer was declared a health priority in the country.

In September 1999, we produced a draft protocol concerning cervical screening in Peru, incorporating the use of visual inspection of the cervix after the application of acetic acid (VIA) in combination with two laboratory-based tests: Hybrid Capture II to detect Human Papillomavirus (HPV) DNA and liquid-based cytology. In the same month, the Bill and Melinda Gates Foundation awarded $50 million to the Alliance for Cervical Cancer Prevention for a major new effort to prevent cervical cancer in developing countries. The Alliance is made up of five international organisations: AVSC International, IARC (International Agency for Research on Cancer), JHPIEGO Corporation, PAHO (Pan American Health Organisation), and PATH (Program for Appropriate Technology in Health). PAHO and PATH decided to start a screening

programme in the region of San Martin, Peru, in collaboration with the Ministry of Health of Peru. The intervention would try to screen 80% of women aged 25-49 in the region, to detect cervical lesions using VIA and to immediate treat with cryotherapy. Results from the project should give recommendations regarding of how to set an organised cervical screening programme in the Peru.

2.4. San Martin

The department of San Martin is located in the Amazon forest of Peru. It has a warm and humid climate with average maximum and minimum temperatures of 36°C and 19°C, respectively.

The department is divided into ten provinces: Bellavista, El Dorado, Huallaga, Lamas, Mariscal Caceres, Moyobamba, Picota, Rioja, San Martin and Tocache.

The capital of the department is Moyobamba located in the province of the same name, however the main city is Tarapoto situated in the province of San Martin.

Tarapoto was founded later but has become one of the commercial centres in the Peruvian Amazonian region. It has an airport with daily flights to different cities of the coast and jungle. The Marginal Highway connects the city with the rest of the region and the rest of the country. This highway is not yet paved and rains usually make it a route of mud holes so journeys can become very tedious and long.

The population of the department of San Martin was estimated to be 667400 in 1997, based on data from the 1993 National Census [205]. The birth and mortality rates of the department were of 26.8 and 5.3 per 10^3 with a density population of 13 inhabitants per square km. It is one of the few departments where the male/female rate is over one (1.14). Around 61% of the population live in urban areas, however, after 1993 when the leaders of the two main terrorists groups were captured and control over drug traffic improved, migration into rural areas increased. Migrants move to work in agriculture (61.5% of employed population). It should be

noticed that these migrants represent a seasonal mobile population. The illiteracy rate is 12.5%.

San Martin is one of the departments where important Peruvian ethnic groups live, either under their original lifestyle or with a mixture of a modern and aboriginal life. According to the Census of 1993, of 65 ethnic groups registered all over the country, three of them have communities living in the region of San Martin: the Aguarunas, the Lamas-Chachapoyas and the Chayahuitas and are among the ten largest ones in Peru. The Aguarunas population is estimated to be around 45,137 inhabitants. They are located in the departments of Amazonas, Cajamarca and Loreto and in the provinces of Rioja and Moyobamba in San Martin. There are approximately 22,513 Lamas-Chachapoyas, composed of 51 different communities distributed over the provinces of Bellavista, El Dorado, Huallaga, Lamas, Picota, San Martin and Tocache, all in the department of San Martin. The Chayahuitas do not communicate with any other group in the Amazonian region. According to the 1993 census, there were 13,717 Chayahuitas mainly in the departments of Loreto and San Martin.

The health region of San Martin (Direccion Regional de Salud, DIRES), representative of the Ministry of Health, has headquarters in the city of Tarapoto. It has a very well structured and implemented network that offers services to inhabitants including vaccination against endemic diseases of the area. Health posts and centres refer cases and send collected specimens to larger hospitals using network facilities. One of the main problems of the area is transport. Heavy rains sometimes make it impossible to travel from one town to another either by river or by road. This causes serious delays on sending specimens and referring patients and on receiving laboratory materials and results. One reason for inhabitants neglecting referral and increasing complications and losses to follow up is the poor transport particularly in the rainy season.

Cytological screening has been on going for several years, but results have never been published. Women seeking attention at any health facility are

educated about cervical cancer and offered cytological screening every year. Smears are repeated on previously screened women of all ages.

San Martin has four cytology laboratories; the principal one is located in Tarapoto and covers the provinces of Bellavista, El Dorado, Huallaga, Lamas, Mariscal Caceres, Picota and San Martin. There is one in Moyobamba and one in Rioja, and a fourth one in Tocache covering the provinces of the same names.

In October 2000, a well-known Peruvian cytopathologist visited the first two laboratories and complained that neither laboratory kept adequate records making it impossible to evaluate performance. The laboratories had no pathologists and only the one in Tarapoto had adequate installations, but even in Tarapoto, the risk of contamination needed to be eliminated.

As a consequence of this report, technical personnel of the Tarapoto laboratory were sent to INEN's cytology laboratory for retraining. DIRES San Martin has made efforts to hire a pathologist to be in charged of the pathology of the region, but they have been unsuccessful.

According to unpublished hospital statistics, a total of 93 cases of invasive cancer of the cervix (most in advanced stages) in women of San Martin, were diagnosed and treated at INEN in the period 1997-2000. If it were assumed that they are all the incident cases from the department of San Martin, then the crude incidence rate of cervical cancer would be 9 per 10^5 women per year. Moreover, if assumed that at most 30% of cases will reach INEN, this rate will increase up to 30, a very high one.

An under-registration rate of 52% has been estimated for mortality collection in the department of San Martin. This and the fact that San Martin does not have a cancer registry leave us with no valid statistics of deaths due to cervical cancer.

Gage *et al* [9,10] reported a study concerning the follow-up of women with abnormal cytology in the region. From January 1999 to April 2000, a total of 233 abnormal smears were registered on cytological registries of the region. Information recalled showed that only 46 (25% of eligible women)

were diagnosed and treated adequately and the rest were lost to follow-up with or without diagnosis. Six of them died of invasive cervical cancer. This study confirmed that one of the main causes of failure of cervical screening programs in difficult settings is lack of treatment and follow-up.

In summary, even though cytological screening has been going on in San Martin in a routine way, it has been ineffective.

2.5. The TATI project

By the end of 1999, two partners of the Alliance for Cervical Cancer Prevention, PAHO and PATH, decided to run a "see and treat" intervention in cervical cancer somewhere in Peru (Tamizaje y Tratamiento Inmediato de Lesiones Cervico-uterinas, TATI project). By February 2000, PAHO and PATH in co-ordination with the Ministry of Health of Peru (MINSA) decided to set up the project in the region of San Martin.

The primary objectives of the project were:

1. To assess the accuracy, cost effectiveness, safety and acceptability of screening and immediate treatment of lesions of the cervix uteri.
2. To develop capacity at primary and secondary level for management of dysplasia and invasive cancer so that cases can be timely resolved at the lowest possible cost to patients and the system. The hope was to screen 80% of the eligible population within three years.

Despite the fact that the more than 50% of the cervical cancers occur in women over 49 years of age in Peru, as showed by data from the cancer registries of Trujillo [200] (55%) and Lima [201] (58%), the gynaecological cancer control programme of the government states that all women aged 30-49 should be screened at least once in their life. However in this intervention, because the average age at first intercourse in the region is 17 as compared to the national average of 19 years, it was decided that women aged 25-29 would be included. Therefore, the target population was 91,413 women aged 25-49 residing in San Martin. There was no population list of

the region, but major efforts were made to invite most women in the area and a list of those invited that refused to participate was kept at each health centre involved.

Women were invited to participate during routine visits to health centres, or through meetings within local women's organizations, or when health campaigns are carried out in their communities. Free (of cost) screening and treatment were offered to women who:

(i). had not been previously diagnosed with cervical cancer;

(ii). had not had a hysterectomy or conization of the cervix;

(iii). have had sexual intercourse at any time in their life; and

(iv). were not pregnant (self-reported) at time of screening.

Women who accepted to participate signed an inform consent, in which the examination with visual inspection after application of acetic acid (VIA) and the following magnified visual inspection using an AviScope™ (VIAM) in case of testing positive were fully explained.

Sixteen lead health centres were designated across the 10 health networks (one per province) in which the region of San Martin is divided. One general doctor and at least one midwife were selected from each of these lead health centres and became a TATI team. Five lead health centres only included midwives. In these centres, doctors from other centres came to examine and treat VIA positive women, as frequently as necessary.

TATI teams were trained in a special course in Tarapoto in November 2000. Doctors and midwives received lectures on gynaecological aspects related to pre-cancerous lesions and cervical cancer, on how to perform gynaecological exams, VIA and collecting Pap smears, and in the protocol to be followed in the project. Doctors were also trained to perform VIAM and cryotherapy and to take punch biopsies. Doctors and midwives were able to practice on anatomical models first, and then on a selected group of women, who were invited to be re-screened and were transported together with local health personnel into Tarapoto because of having had a positive

cytology in the previous year. Previously developed data collection forms were discussed among participants during the course.

Table 2.5.1. TATI teams across the region of San Martin.

Health Network	Lead Health Centres	Doctors	Midwives
Bellavista	HR Bellavista	1	1
DIRES	C Materno Perinatal	1	2
El Dorado	HR San Jose de Sisa	1	1
Huallaga	HR Saposoa	0	1
	CS Sacanche *	1	0
Mcal. Caceres	CS La Merced	1	1
Moyobamba	CS Jepelacio	0	1
	CS Lluyllucucha *	1	2
	CS Soritor	1	1
Picota	HR Picota	1	1
Rioja	CS Nueva Cajamarca *	1	1
	CS Nueva Rioja	0	1
	PS San Juan Soritor	0	1
Lamas	HR Lamas	0	1
San Martin	CS Tabalosos *	1	1
	CS Pongo del Caynarachi *	1	0
	CS Juan Guerra	0	1
Tocache	HR Tocache	1	2
Total	**16**	**12**	**19**

* Doctors went to other health centres to examine/treat positive VIA women.

After the course TATI teams went back to their towns and put the project in practice. After two months, a new meeting to discuss strong and weak

points of the protocol was held in Tarapoto; modifications were made to data forms and the project continued. Midwives screened patients as part of their daily professional activities. Women were invited to participate when visiting lead health centres, or by encouragement through women's community groups, or during health campaigns. Those accepting to participate were asked to read and sign an informed consent, where all procedures were explained. Midwives performed a gynaecological exam and VIA, and took a Pap smear before applying acetic acid. Women found positive on VIA, were seen by a TATI doctor who performed VIAM. Doctors treated with cryotherapy (after taking a punch biopsy of the lesion) women found positive on VIAM who signed another informed consent, or referred them to colposcopy. Two gynaecologists especially trained, performed colposcopy, treated women with cold conization, LEEP, or hysterectomy; or referred them to INEN (if other treatment was required).

2.6. Development of protocol

2.6.1. Initial plan

In September 1999, we produced a draft protocol concerning cervical screening in Peru, incorporating the use of visual inspection of the cervix after the application of acetic acid (VIA) in combination with two other tests: Hybrid Capture II to detect human papillomavirus (HPV) infection and liquid-based cytology (LBC). Colposcopy was to be offered to every woman testing positive on VIA, those negative on VIA but positive either on LBC or HPV testing, and in a random sample of those negative in the three tests. The aim was to detect high-grade squamous intraepithelial lesions (HSIL). Histologically was considered the gold standard for diagnosis. The protocol was to investigate the best cervical screening strategy in three different areas of Peru: a very deprived district in Metropolitan Lima, the province of Huamanga in Ayacucho (Sierra) and Iquitos in the Amazonia of Peru. This study would also compared HPV DNA prevalence and other risk factors for cervical cancer in the three

geographic regions of Peru.

2.6.2. Collaboration with PAHO and PATH

Funds were searched among different international organisations. As explained earlier in this document, PAHO and PATH, two partners of the Alliance for Cervical Cancer Prevention in co-ordination with the Ministry of Health of Peru, decided to run a "see and treat" intervention in cervical cancer in the department of San Martin (TATI project). PAHO and PATH decided to allow us to incorporate our protocol in the TATI project, in this way, we were able to use the infrastructure already in place in the region and the facilities implemented by the TATI project. Nevertheless, our original protocol was modified in order to be nested within the TATI project. The protocol was to be set up only in San Martin, magnified visual inspection was added to the screening scheme and conventional cytology.

PAHO was to be responsible for the overall "see and treat" intervention. PATH was to implement recruitment strategies as to achieve the screening goals, and we were to be in charge of the research protocol, enrolling 5,000 women with VIA, LBC and HPV testing. Our responsibilities included training, implementation and monitoring of LBC and HPV testing, as well as, organising histology to be processed at the INEN pathology laboratory, and the analysis of the cohort of the first 5,000 women screened with all tests.

3. OBJECTIVES

3.1. Aim of the study

To evaluate the sensitivity, specificity, acceptability and feasibility of various combinations of four different techniques for cervical screening.

The techniques are:

i. Visual Inspection after Acetic acid application (VIA) by a midwife (or nurse) without magnification;

ii. Combined use of unaided VIA and VIA by a doctor using an AviScope™ device (VIAM), in those judged to be positive by means of unaided VIA;

iii. Liquid-based cytology (LBC) using AutoCyte-Prep®'s manual system (Tripath); and,

iv. Human Papilloma Virus (HPV) testing using Hybrid Capture II (Digene).

3.2. Main Objectives

1. To estimate the overall sensitivity and specificity of various combinations of screening tests in San Martin, Peru, for detection of high-grade squamous intra-epithelial lesions (HSIL) on histology.
2. To estimate the relative sensitivity of HPV testing (Hybrid Capture II) and liquid-based cytology in women with acetowhite lesions on unaided VIA.
3. To evaluate the effect of requiring a doctor's AviScope™ confirmation of unaided VIA on the sensitivity and specificity of this screening technique.
4. To train a laboratory in Lima to be able to carry out liquid based cytology using the manual system of AutoCyte-Prep®.

3.3. Secondary Objectives

1. To estimate the age-specific prevalence of HSIL and occult cervical cancer among women in San Martin, Peru.
2. To estimate the prevalence of different types of oncogenic HPV infections in San Martin, Peru.
3. To establish a screening network involving the Peruvian Ministry of Health, the National League against Cancer, NGOs working on behalf of poor women, community women's groups, the National Association of Midwives, colposcopists and pathologists that would be well placed to advise and oversee a national cervical screening program.
4. To investigate the possible role of ethnicity, diet, smoking and reproductive history as cofactors in the aetiology of high-grade cervical lesions in women exposed to oncogenic HPV.
5. To determine the most cost-effective combination of screening tests for use in a developing country.
6. To ascertain the infrastructure needed to implement a national screening programme with a goal of 75% coverage of women before the age of 50 years.

These secondary objectives, despite being part of the study, will not be assessed in this thesis.

3.4. Hypothesis

A two-stage screening process, in which all women have a VIA and those with any suspected lesion receive an additional test (either LBC or HC-II), is a cost-effective approach to cervical screening in San Martin, Peru.

4. METHODS

4.1. Basic design

4.1.1. Population

Women enrolling into the TATI project were invited to participate in this study. The first 5,600 women who, after being offered additional screening tests (HPV testing and LBC), signed an informed consent for additional cervical samples, were included in the study.

4.1.1.1. Inclusion criteria

As in the TATI project women were included if:

a. They were between 25 and 49 years of age, and;

b. They have had sexual intercourse at any time in their life, and;

c. They were not pregnant (self-reported) at the time of screening, and;

d. They had not had a hysterectomy or conization of the cervix, and;

e. Signed the corresponding informed consent.

4.1.1.2. Exclusion criteria

Women were excluded if:

a. Their samples for LBC and HPV were collected but were not examined with VIA by a midwife,

b. They had VIA but both LBC and HPV samples were inadequate or missing,

c. They were first evaluated during the TATI training course in November 2000 (women were invited if abnormal on previous cytology),

d. They were screened with VIA within previous 12 months and tested positive, or;

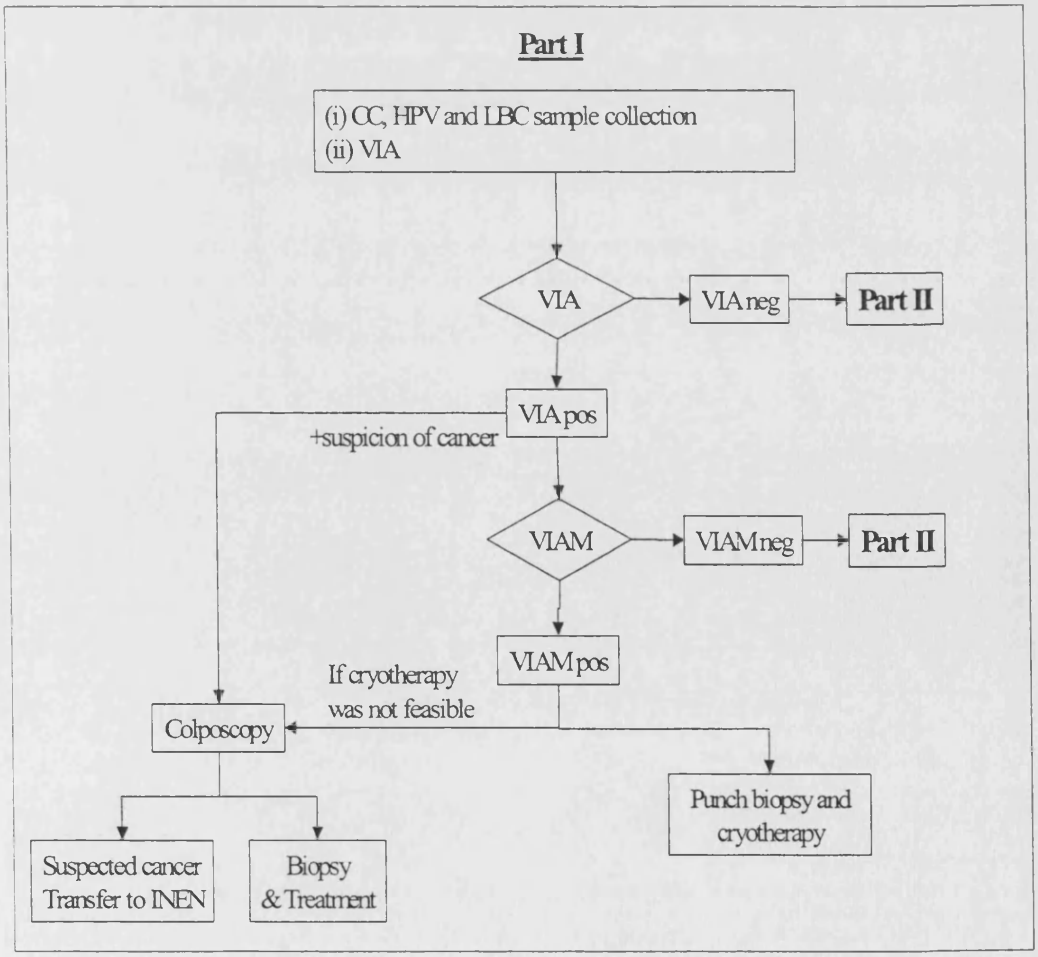
e. They came into clinics because of having severe symptoms indicative of gynaecological cancer; and the midwife decided they were not suitable for screening.

4.1.2. First part of the protocol

This is a nested screening study within the TATI project in the region of San Martin, Peru.

The first part of the protocol is presented in Figure 4.1.2.1.

Figure 4.1.2.1. First part of the protocol.



All women participating in the study were screened with four screening tests. Midwives performed first a gynaecological exam and visualised the cervix after introducing a speculum.

A cervical sample for conventional cytology (CC) (Papanicolaou smear) was collected by means of an Ayre spatula, by rotating it 360 degrees in the area of the squamocolumnar junction, smearing and fixing the cells from the spatula on a glass slide. Samples were sent to cytology laboratories within a month of collection.

A second sample for liquid-based cytology (LBC) was collected using a Rovers-Cervex® brush. The brush was inserted into the endocervical canal and rotated five times clockwise. The brush head was transferred directly into a vial containing CytoRich® Preservative Liquid and kept in a regular refrigerator (4°C).

A third sample for HPV DNA testing was collected using a Digene cervical brush. The brush was inserted 1 to 1.5 cms. into the cervix and rotated three times in anti-clockwise direction. Collected samples were immediately stored in tubes containing Digene Sample Transportation Medium (STM) and kept in a regular refrigerator (4°C).

Midwives then performed VIA. They first applied a solution of acetic acid (nominally 5%), and after waiting for a minute, examined the cervix for acetowhite areas close to the squamocolumnar junction. VIA was considered positive if any acetowhite lesions were observed in or close to the transformation zone, or negative otherwise. Midwives referred women to the doctor if VIA was positive or directly to the colposcopist if they found evidence of suspected invasive cervical cancer. However, in some cases midwives referred women straight to colposcopy because they detected large acetowhite lesions or the general doctor would not be available for a long period.

Doctors performed VIAM using an AviScope™ device (4x magnification, green light). VIAM was considered positive if acetowhite lesions were observed, and negative otherwise. Women testing positive were offered

immediate treatment with cryotherapy after being asked to sign another informed consent to be biopsied and treated. Punch biopsies of the compromised areas of the cervix were first taken on those who accepted the treatment. If lesions covered more than 75% of the cervix, or involved the endocervical canal, or were clearly invasive cancer, women were referred to colposcopy.

Gynaecologists performed colposcopy, took biopsies and treated women with LEEP, cold conization or hysterectomy as appropriate, and referred to INEN those suspected of having invasive cancer.

Biopsies were stored in formol at 20% and sent regularly to the INEN's pathology laboratory in Lima.

Women who tested positive on VIA and were screened in any of the health centres where a doctor was not available, were given an appointment in accordance with the doctor's schedule. Women who did not attended their VIAM appointment, were contacted by health centre personnel and were given new appointments.

Women who tested negative for VIA or VIAM were told to come back for another screening in three years and to return or contact the health personnel for the other screening results in two months. If results were available before and one was positive, women were contacted by health personnel (see below).

4.1.3. Second part of the protocol

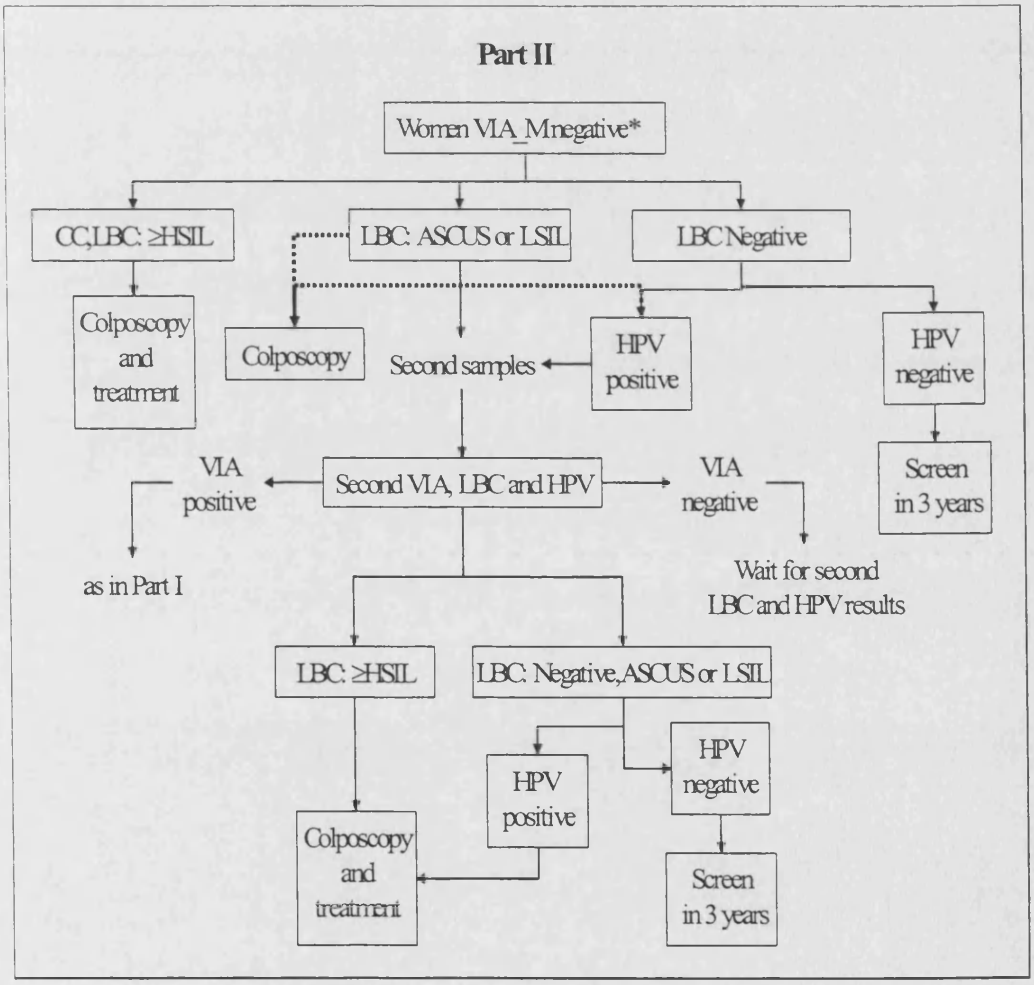
The second part of the protocol is presented in Figure 4.1.3.1.

Women who tested negative on visual methods and return to the health centre for their other test results, were told to come back in three years if they tested negative on LBC and HPV. Those who did not come back for results but were negative on all screening tests were not traced again; while those testing positive on either LBC or HPV were visited by health personnel, usually midwives, who explained the results and encouraged

women to come to the health centre to discuss their management options with the TATI midwives.

Women who were not already treated but whose cell samples tested positive on either LBC or Hybrid Capture could be managed under two options: either to have a second screening within 6 to 12 months from the first one ("no colposcopy option"), or to have a colposcopy ("colposcopy option"), as shown in Tables 4.1.3.1.a, 4.1.3.1.b, and 4.1.3.2.

Figure 4.1.3.1. Second part of the protocol.



* VIA_M negative = VIA negative or VIA positive but VIAM negative.

As conventional cytology was not part of this study, it was only taken into account if a high-grade cervical lesion was reported, when similarly to LBC, women were referred to colposcopy and adequate treatment.

Most women were managed under the "no colposcopy" option, since there were only two centres offering the exam, and those with HSIL (or worse) on either LBC or CC already filled the colposcopists timetables.

Table 4.1.3.1.a. Management according to results of testing first samples "no colposcopy option" in women with a negative VIA(M)[1].

LBC	HPV testing	
	HPV +	HPV -
HSIL[2]	Colposcopy and treatment	Colposcopy and treatment
LSIL or ASCUS	6-12 month second tests	6-12 month second tests
Negative	6-12 month second tests	Screen in 3 years

1 For women not treated with cryotherapy at initial visit.
2 HSIL or more on LBC or CC.

Table 4.1.3.1.b. Management according to results of the follow-up sample in women with a negative VIA on following screening (regardless of their results from first samples) under the "no colposcopy option" [1].

LBC	HPV testing	
	HPV +	HPV -
HSIL [2]	Colposcopy and treatment	Colposcopy and treatment
LSIL, ASCUS, or NEGATIVE	Colposcopy and treatment	Screen in 3 years

1 For women not treated with cryotherapy at initial visit.
2 HSIL or more on LBC or CC.

Those under the "colposcopy option" were managed as presented in Table 4.1.3.2.

Table 4.1.3.2. Management according to results of test samples taken on initial visit under the "colposcopy option" [1].

LBC	HPV testing	
	HPV +	HPV -
HSIL [2]	Colposcopy	Colposcopy
LSIL or ASCUS	Colposcopy	6-12 month second tests
Negative	Colposcopy	No follow-up

1 For women not treated with cryotherapy at initial visit.
2 HSIL or more on LBC or CC.

Health personnel visited several times women requiring second screening, which was intended to happen within 6 to 12 months of initial screening, but in many cases it only took place after 12 months.

Second screening included collection of samples for CC, LBC and HPV testing, and a second VIA exam performed by a midwife. If this second VIA was positive, women were referred to VIAM or directly to colposcopy, following the same guidelines as in the first part of the protocol. If either VIA or VIAM was negative, women were told to come back in three weeks for their other results.

As explained on Table 4.1.3.1.b., women with HSIL (or worse) on LBC or HPV positive were also referred for colposcopy and further treatment. Those testing negative on HPV and having lesions no worse than LSIL, were given their results and told to come back in three years. An external review of LBC second samples classified as LSIL when the corresponding HPV test was negative was organized to ensure women with these second results were free of disease.

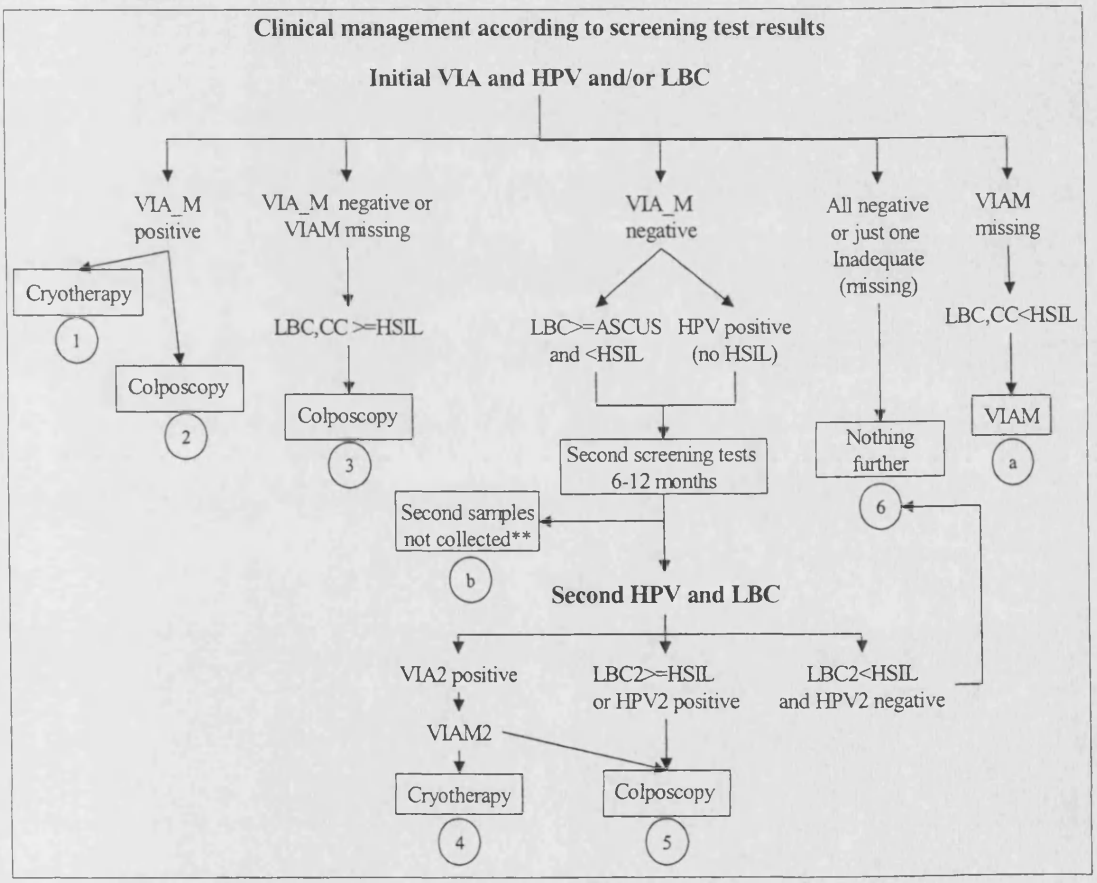
A summary of the clinical procedures applied to women according to their screening results is presented on Figure 4.1.3.2. In this figure, women have been grouped in six clinical management options according to the type of treatment of evaluation they required. The groups are:

1. VIA_M cryotherapy: women VIA positive and VIAM positive who required cryotherapy;

2. VIA_M colposcopy: women with VIA positive referred to colposcopy, or referred to VIAM which was also positive and referred to colposcopy;

3. HSIL colposcopy: women VIA negative, or VIA positive but VIAM negative or missing who had HSIL on LBC or CC, and so required colposcopy;

Figure 4.1.3.2. Clinical management according to screening test results.



* VIA_M is a combination of VIA and VIAM screening tests.
** Or second samples were collected but results were not available at time of analysis.

4. Cryotherapy second screening: women VIA negative, or VIA positive but VIAM negative (untreated) with second screening whose second

VIA was positive, had VIAM which was also positive and were treated with cryotherapy;

5. Colposcopy second screening: women VIA negative, or VIA positive but VIAM negative (untreated) with second screening whose second VIA was positive and were referred straight to colposcopy, or their second VIA was positive, had VIAM also positive and instead of being treated with cryotherapy were referred to colposcopy; or those who had HSIL or worse on second LBC or had their second HPV test positive; and,

6. No further evaluation: women testing negative in all first tests or inadequate in one and negative in the others; or those VIA negative or VIA positive but VIAM negative (untreated) whose second VIA was negative or positive but their second VIAM was also negative and their LBC was less than HSIL and their HPV was negative, and so did not require further evaluation.

It is worth noticing that there are two groups of women with incomplete screening:

a. Women who were referred to VIAM after VIA, but were not examined with VIAM; and,

b. Untreated women VIA negative or VIA positive but VIAM negative who required second screening but have not had the second screening so far, or whose second screening tests results are not available yet.

4.2. Laboratory techniques and data handling

4.2.1. Sample handling

4.2.1.1. Collection and storage

Collected CC slides were sent to the corresponding laboratory using the health network connections (sometimes courier or official cars) once a month, and so results were not available for 2-3 months after collection. Since the start of 2002, CC results are available within 3 weeks of collection, since slides are sent more frequently to the cytology laboratories.

TriPath and Digene recommend to maintain collection kits and collected samples at less than 30°C. But temperatures in the region can easily reached 36°C, especially in the dry season (July to December). LBC and HC-II collection kits were stored in a room with full air-condition in the administrative TATI office in Tarapoto. Once a month, between 50 and 100 HPV and LBC kits were delivered to each health centre, according to the refrigerated storage space within their facility. Deliveries were done in three trips, one covering the north (usually took two days), one the centre and one the south of the region. Trips were done in the TATI office car, keeping the air-conditioning on maximum, using "The Marginal", main highway that connects all provinces in the region. Unfortunately only 40% of the highway is asphalted and trucks get easily trapped in the middle of the route for hours, and in the rainy season, small lagoons are formed making it impossible for cars to go further, therefore a distance of 100 km can becomes a twelve-hour journey.

4.2.1.2. Transport of collected samples

Collected samples of LBC and HC-II samples were properly labelled, closed and secured with masking tape by midwives or their assistants, and stored in refrigerators in the lead health centres. Once a week, samples

were sent to the administrative TATI office in Tarapoto. Adequate transport of samples was crucial.

Samples were arranged in termical boxes, which then were filled with ice and closed. Termical boxes were then labelled and secured with masking tape. A list (see Appendix) containing codes and names of sample owners was sent together with the boxes.

Samples were transported in three different ways:

a. Boxes were collected when additional empty collection kits were delivered to health centres, and taken to Tarapoto in the TATI car.

b. Boxes were taken by the midwife or her assistant if they were going into Tarapoto for some meeting, or were bringing patients.

c. Boxes were sent by courier service directly to the TATI office. Couriers guaranteed the arrival of boxes to their destination but not the time of arrival.

Once boxes arrived at the TATI office, sample tubes were immediately dried (depending on how long the journey was, the ice would have already melted), re-labelled according to the list added to the box, and stored in a regular refrigerator (4°C).

LBC collected samples were sent to Lima twice a month, while HPV samples were sent to London every two months.

LBC results were available within 2-3 weeks of screening, and the HPV ones after 3-4 months of screening. Results were sent by fax or personally delivered to the health centres.

4.2.2. Liquid-based cytology

Conventional cytology was not considered part of this study; nevertheless it was offered to all participants, following regulations of the Ministry of Health. It was processed and read in three different laboratories.

Liquid-based cytology was performed in the cytology laboratory of INEN using the AutoCyte-Prep® manual system (TriPath). In this process, the

sample is thoroughly mixed and disaggregated by vortexing, and excess white cells, blood artifact; bacteria and debris are removed by density reagent centrifugation. Cell pellets are suspended in dilutent, and cervical material is sedimented onto specially coated slides. These slides are stained with the usual Papanicolaou stain used by the laboratory.

These LBC slides were then read by cytotechnicians, and reviewed by a cytopathologist.

Table 4.2.2.1. Classification used for LBC results.

Liquid-based cytology result		
- Inadequate	- ASCUS	- Moderate dysplasia
- Normal	- AGUS	- Severe dysplasia/ Carcinoma in situ
- Inflammation	- Condyloma/HPV	- Invasive carcinoma
	- Mild dysplasia	- Adenocarcinoma

Results were classified using the classification show in Table 4.2.2.1. For the analysis condyloma/HPV and mild dysplasia were merged into low-grade squamous intraepithelial lesions (LSIL) and moderate dysplasia and severe dysplasia/carcinoma in situ into high-grade squamous intraepithelial lesions (HSIL).

The same classification was used for reporting CC results.

4.2.3. Hybrid-Capture II

HPV DNA testing was performed, by Dr. Philip Londesborough in Dr. Linda Ho's HPV laboratory of Cancer Research UK, using Hybrid Capture II (HC-II, Digene Corporation). This technology is a signal amplified hybridisation antibody capture microplate assay that utilises chemiluminescent detection.

Specimens containing the target DNA are denatured and hybridised with HPV RNA probe cocktail (13 full-length RNA probes recognising

oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). The resultant RNA:DNA hybrids are captured onto the surface of a microplate well coated with antibodies specific for RNA:DNA hybrids. Immobilised hybrids are then reacted with alkaline phosphatase conjugated antibodies specific for the RNA:DNA hybrids, and detected with a chemiluminescent substrate. Several alkaline phosphatase molecules are conjugated to each antibody. Multiple conjugated antibodies bind to each captured hybrid resulting in substantial signal amplification. As the substrate is cleaved by the bound alkaline phosphatase, light is emitted which is measured as light units on a luminometer. The intensity of the light emitted denotes the presence of absence of target DNA in the specimen [206].

Results were given as the ratio of the relative light units given by the test specimen to that given by a 1pg/ml HPV DNA control (RLU/CO) (number of RLUs for a 1pg cut-off).

4.2.4. Data handling

A questionnaire was applied to all women entering the study. Age, ethnicity, education (years at school), province of residence, age at first intercourse, number of sexual partners, parity, abortion, use of contraceptives (method, time), number of Pap smears in life, date and result of the last Pap smear and smoking status (never, ex, current) were collected.

Seven different data forms have been used by the TATI project (see Appendix). The first one registers the variables mentioned above; the second and third one used to report VIA and VIAM results. The fourth one contains results from follow-up visits after cryotherapy. The fifth one is reports the findings during colposcopy. The sixth and seven are lists of collected conventional cytology samples and biopsy specimens to be sent to respective laboratories. For our protocol, a simple page was designed for the collection of additional samples. These lists contain a special sample

code, date of sample taking, name and age of each screened woman, and were stored in a Microsoft Excel file together with corresponding results.

Results from screening tests were sent to the TATI administrative office, as soon as they were available.

Personnel in the TATI administrative office use an information system, especially generated for the TATI project, to input data collected from the different forms. As samples were collected, the TATI information system periodically provided us with a database containing variables relative to our protocol. This database was merged with our Excel worksheet and a complete file with relevant information for this study was obtained.

Data was then stored in a STATA file, which has been used for analysis.

Data cleaning started as soon as data were available. This helped to point out problems of the main TATI database and particularly of the follow-up of screened women. The TATI office was informed of any inconsistency found.

4.3. Statistical methods

4.3.1. Definition of disease

Histology was the gold standard for comparing screening tests (Table 4.3.1.1).

The true histology of any woman whose screening results warranted colposcopy or cryotherapy (according to the above tables), but who did not have a biopsy or colposcopy will be treated as "missing at random" [207] within clinical management groups (Figure 4.1.3.2). Women who tested negative on all screening tests were treated as if they did not have HSIL on histology, even though we can not be certain of that. For this reason, estimates of measures of tests performance should be considered as relative rather than absolute measures of performance.

Table 4.3.1.1. Definition of true positives and true negatives.

True Positives	True Negatives
- Histology HSIL or worse	– VIA_M negative and liquid-based cytology negative or inadequate, and HPV negative or inadequate (but not both inadequate). – VIA_M negative and cytology no worse than LSIL and on follow-up: VIA negative, and cytology no worse than LSIL and HPV negative. – Adequate negative colposcopy (i.e., no visible lesion and no biopsy taken). – Histology no worse than LSIL.

4.3.1.1. Women with undetermined disease status

In summary, disease status was not fully determined in:

- Women with indicated VIAM, which was not performed (unless women had HSIL on cytology and required colposcopy);
- Women referred to colposcopy (after first or second screening tests); which was not performed;
- Women who required second samples, which were not collected, or women who had second samples but results were not available at time of analysis.
- Women with cryotherapy but who were not biopsied;
- Women who had inadequate biopsies (no pathology available).

These women were considered "not fully evaluated" for analysis, and therefore were assumed to be "missing at random".

4.3.2. Variables defined from screening tests for analysis

The variables used in the analysis defined according to first screening tests results are:

a. VIA1: positive if VIA was positive and negative otherwise.

b. VIAM1:

- **Cryo** if VIA was positive and VIAM was positive and cryotherapy was indicated;
- **Colp** if VIA was positive and VIAM was positive and referred to colposcopy;
- **Negative** if VIA was positive but VIAM was negative; and
- **Missing** if VIA was positive and women were referred to VIAM but this was never performed.

c. VIA_M1: a combination of results of VIA and VIAM, defined as:

- **Cryo1** if VIA was positive and VIA referred to VIAM, which was positive and indicated cryotherapy;
- **Colp1** if VIA was positive and VIA referred straight to colposcopy; or, if VIA was positive and VIA referred to VIAM, which was positive and referred to colposcopy; and,
- **Negative** if VIA was negative and VIAM was not indicated nor performed or VIA was positive and referred to VIAM, which turned out to be negative.

d. VIA_MM1: another combination of results of VIA and VIAM, defined as:

- **Cryo1** if VIA was positive and VIA referred to VIAM, which was positive and indicated cryotherapy;

- **Colp1** if VIA was positive and VIA referred straight to colposcopy; or, if VIA was positive and VIA referred to VIAM, which was positive and referred to colposcopy;
- **VIA neg** if VIA was negative and VIAM was not indicated nor performed; and,
- **VIAM neg** if VIA was positive and VIAM was negative.

e. LBC7: including LBC results divided in 7 categories:

- **Neg** if LBC negative or inflammation;
- **ASCUS** if ASCUS or AGUS;
- **LSIL** if condyloma/HPV or mild dysplasia;
- **Moderate** if moderate dysplasia;
- **Severe** if severe dysplasia or carcinoma in situ;
- **Cancer** if carcinoma; and,
- **Inadequate** if samples were insufficient for testing.

f. LBC6: including LBC results divided in 6 categories:

- **Neg** if LBC negative or inflammation;
- **ASCUS** if ASCUS or AGUS;
- **LSIL** if condyloma/HPV or mild dysplasia;
- **HSIL** if moderate dysplasia or severe dysplasia or carcinoma in situ;
- **Cancer** if carcinoma; and,
- **Inadequate** if samples were insufficient for testing.

g. LBC4: including LBC results divided into 4 categories:

- **Neg** if LBC negative or inflammation;
- **LSIL** if ASCUS, AGUS, condyloma/HPV or mild dysplasia;

- **HSIL** if moderate dysplasia or worse; and,
- **Inadequate** if samples were insufficient for testing.

h. LBCRES: including LBC results divided into the 4 categories used to define women requiring second screening:
   - **Neg** if LBC negative or inflammation;
   - **ASCUS** if ASCUS or AGUS;
   - **LSIL** if condyloma/HPV or mild dysplasia; and,
   - **Inadequate** if samples were insufficient for testing.

i. LBCLOW:
   - **Pos**, if ASCUS, AGUS, Condyloma/HPV, LSIL, HSIL or worse on LBC;
   - **Neg**, if negative or inflammation.

j. LBCHSIL:
   - **Pos**, if HSIL or worse on LBC;
   - **Neg**, if negative or inflammation.

k. HPV1:
   - **Pos**, if HPV testing positive (≥1 RLU); and,
   - **Neg**, if HPV testing negative.

l. HPVHVL: using a higher threshold for HC-II:
   - **Pos**, if HPV testing positive (≥4 RLU); and,
   - **Neg**, if HPV testing negative.

m. CCH_O_LB: HSIL on conventional cytology and less than HSIL on LBC:
   - **Pos**, if HSIL on conventional cytology and less than HSIL on LBC; and,

- **Neg**, otherwise.

n. VIA_LBCHG: interaction between VIA and high-grade disease on LBC:

- **Pos,** if VIA1 positive and HSIL or worse on LBC; and,
- **Neg**, otherwise.

o. VIA_HPV: interaction between VIA and HPV testing:

- **Pos,** if VIA1 positive and HPV1 positive; and,
- **Neg**, otherwise.

p. LBHG_HPV: interaction between high-grade disease on LBC and HPV testing:

- **Pos,** if HSIL or worse on LBC and HPV1 positive; and,
- **Neg,** otherwise.

The variables defined according to required second screening tests (after 6 to 12 months of first samples of LBC and HPV) are:

q. VIA2, defined as:

- **Pos**, if VIA positive and referred to VIAM; or,
- **Neg**, otherwise.

r. VIAM2:

- **Pos** if VIA2 was positive and VIAM2 was positive;
- **Negative** if VIA2 was positive but VIAM2 was negative; and

s. VIA_M2: a combination of second results of VIA and VIAM:

- **Cryo2** if VIA2 was positive and VIA2 referred to VIAM2, which was positive and indicated cryotherapy;
- **Colp2** if VIA2 was positive and VIA2 referred straight to colposcopy; or, if VIA2 was positive and VIA2 referred to VIAM2, which was positive and referred to colposcopy; and,

- **Negative** if VIA2 was negative and VIAM2 was not indicated nor performed or VIA2 was positive and referred to VIAM2, which turned out to be negative.

t. LBC2, defined as:

- **Pos**, if HSIL or worse on second LBC; and,
- **Neg,** if Negative, inflammation, ASCUS, AGUS, Condyloma/HPV, or LSIL on second LBC.

u. HPV2, defined as:

- **Pos**, if second HPV was positive; and
- **Neg**, if second HPV was negative.

v. SECSCR, defined as:

- **Cryo2,** if VIA_M2 was Cryo2;
- **Colp2,** if VIA_M2 was Colp, or LBC2 was HSIL or worse, or HPV2 was positive;
- **Neg**, if VIA_M2 was negative, LBC2 was less than HSIL and HPV2 was negative; and
- **Missing,** if any of the three results were missing or second screening was required but not performed.

w. HSIL or disease status defined as:

- **HSIL,** if histology was HSIL or worse;
- **No HSIL,** as True Negatives are defined in Table 4.3.1.1; and,
- **Missing,** if women were "not fully evaluated".

4.3.3. Statistical analysis

All analyses were performed using STATA, version 8.0.

4.3.3.1. Descriptive statistics

A summary of the study population characteristics is presented, including demographics, reproductive and sexual factors, and history of previous cervical screening.

4.3.3.2. Results from screening tests

Results from screening tests are tabulated; those of conventional and liquid-based cytology are detailed.

Positivity rates of each screening test are tabulated. The positivity rate of VIA_M is calculated as a weighted measure taking into account the corresponding VIA result. Positivity rates of CC are tabulated only for detecting high-grade disease, as this is the only case when they affect clinical management (see 4.2.2 above). Two thresholds are considered for LBC: ASCUS or worse (LBCLOW) and HSIL or worse (LBCHSIL) and two for HPV (≥1 RLU and ≥4 RLU).

The dependence of each screening test on age and on place of screening (health center) is evaluated using Score tests for trends or Chi-square tests, as appropriate. To determine the association between VIA_M and age or health center, weighted proportions and trends across age groups or health centers are evaluated using Wald tests obtained through weighted logistic regression models, which account for the original VIA result.

Positivity rates of different combinations of screening tests are then tabulated: each of VIAM, LBC, CC (only if HSIL or worse) and HPV within VIA positive women, within VIA negative women, within VIAM negative women (being VIA positive first), and within VIAM positive (being VIA positive first).

4.3.3.3. Estimating sensitivity and specificity of screening tests

Figure 4.3.3.3.1. Algorithm used for the estimation of missing data and undetermined disease status.

Algorithm for estimation of missing data and undetermined disease status

1. ***"Fill in missing VIAM results"***
   a. Estimate the probability of different results on VIAM conditionally on results of other tests.
   b. If a woman has VIA positive, but no VIAM or final diagnosis, replace her with three pseudo observations, one with each of the three possible VIAM results.
   c. Assign weights to the pseudo-observations equal to the estimated probability of the observation given the other test results. Assign weight one to all original observations.
2. ***"Fill in missing second stage tests results"***
   a. Estimate the probability of different results (discharge, treat or refer) following second stage testing conditional on first stage results using data from women who have results from all three (VIA_M, LBC, HPV) second-stage results.

      (NB: Exclude women who are VIAM2 positive but HPV2 or LBC2 unknown).
   b. If a (pseudo)-observation ought to have had second-stage screening, but it has yet not been done or the results are not yet available and if the woman has not had a final diagnosis, replace observation with three observations.
   c. Assign weights to the new pseudo-observations equal to the estimated probability of the observation given the first stage results.
   d. Assign weight one to all observations not created in step 2b.
3. ***"Fill in the missing disease status"***
   a. Estimate the probability of high-grade disease given the available screening results. This must be done taking into account the different chances of being fully evaluated depending on the screening results. We do this by identifying 7 "clinical management groups" and assume that within each group, the chance of being evaluated is independent of the true disease status. Observations in each clinical group are weighted by the inverse probability of being evaluated. The probability of high-grade disease is then estimated from women who were fully evaluated.
   b. If the final diagnosis is missing, replace the observation by two pseudo-observations: one with HSIL on final diagnosis and the other without HSIL.
   c. Assign weights to the observations as follows:
      - weight one for any woman with known final diagnosis;
      - weight equal to the estimated probability of the pseudo-observation using the weighted model in 3a.
   d. Multiply the weights from 1c, 2c, and 3c together to get a final weight.
4. ***Form tables of pseudo-observations weighted by the weights from 3d.***

Overview

Sensitivity, specificity and positive predictive values could not be estimated directly (by usual definitions) because of missing data regarding screening tests and lack of disease status.

Figure 4.3.3.3.1 details the algorithm used to estimate sensitivity, specificity and positive predictive value of screening tests.

VIAM was not performed in a number of women who tested positive on VIA and were referred to VIAM; and second samples were not collected on women who needed them, or were collected but the results were not available at time of analysis. Allowance for these missing data was made before calculating measures of tests performance. Our approach was to estimate the distribution of the missing values.

Once second screening tests results were estimated, women were re-allocated into one of seven clinical management groups (strata); six were described in Figure 4.1.3.2: two of women treated with cryotherapy (one after initial screening and the other after second screening), three of women referred to colposcopy (two after initial screening and one after second screening tests), one of women who did not required further evaluation (either after first screening tests or after second screening tests); and the seventh one composed of fully evaluated women (with histology) despite incomplete screening (for instance, women with pending VIAM who underwent colposcopy instead).

Despite the fact that women testing negative in all tests either on first or second samples, were considered free of disease; verification bias was introduced because only few women met these criteria or underwent colposcopy or had histology. The rest of women were considered "not fully evaluated".

The probability of having been fully evaluated depended on the seven evaluation strata, as for instance; a woman in group 3 (needing colposcopy because of HSIL on cytology), was more likely to have a colposcopy that one requiring colposcopy after second set of screening tests. Therefore, the

probability of disease was estimated based on the evaluation strata for different screening tests or combination of screening tests.

Dealing with VIAM missing data

A multinomial logistic model was fitted to estimate the possible results of VIAM using the other screening tests results (LBC and HC-II) (Figure 4.3.3.3.1, step 1). The predicted probabilities of having one of three possible results of VIAM: positive needing cryotherapy, positive needing colposcopy and negative were obtained. Data of women with missing VIAM were then expanded three times; so each of these women had one record with each of three possible results. Weights were then generated based on the predicted probabilities obtained from the multinomial logistic model and were assigned to each of three records of women with missing VIAM results. Women with complete data were given weight one. Thus, a woman could have three records corresponding to the three possible results on VIAM but sum of weights for each woman was always one.

Dealing with second screening missing data

A multinomial logistic model was fitted to estimate the possible results of second screening tests using the results of first LBC and first HPV testing (Figure 4.3.3.3.1, step 2). All women with a second test were VIA_M negative. The predicted probabilities of having one of three possible combinations of second screening results: positive needing cryotherapy (VIA_M2 positive indicating cryotherapy), positive needing colposcopy (VIA_M2 positive referring to colposcopy, or HSIL or worse on LBC2, or HPV2 positive) and negative (VIA_M2 negative, less than HSIL on LBC2 and HPV2 negative), were obtained. Data of women with missing second screening were then expanded three times, yielding one record for each of three possible results. Weights were then generated based on the predictive values obtained from the multinomial logistic model and were assigned to each of three records of women with missing second screening results.

Again, the weights for women who needed second screening and had complete second screening tests and for women who did not need second screening tests results were the unity. New weights were defined as the product of the first and second set of weights. Therefore, one woman could have one, three or five records (five for instance if she had VIAM pending and LBC LSIL on the first screening test with no second results), but only a fraction of her had a particular second screening set of results, and so was counted as one person (weights from estimating VIAM results were combined with these ones, and their sum was the unity).

Using these estimated second screening results, the clinical management groups were assigned to each record and related variables were updated.

Estimating disease status

Disease status was estimated using empirical estimation and weighted logistic regression modelling (Figure 4.3.3.3.1, step 3).

Data of women with undetermined disease status were then expanded, so each had one record with high-grade disease and one with free of disease status. Weights were then generated based on the probability of having disease given a history of screening test results. The weights for women who were fully evaluated were the unity.

The probability of having disease on those "not fully evaluated" was estimated by means of:

a. Empirical estimation: using the proportion of women with high-grade disease (who were actually fully evaluated) within different combination of screening tests results (VIA_M1 x LBC x HPV levels). This proportion was treated as an empirical predictive value and was used to generate the first weight (p_1) for those with undetermined disease status.

b. Fitting two logistic regression models using the screening tests results: the first on women who did not require second screening and

the second one on those who needed second screening, the predictive values generated from both models were combined to obtain the second weight (p_2) for women with undetermined disease status.

c. One logistic regression model using the screening tests results of all women in the study. These predictive values were used to calculate the third weight (p_3) for women with undetermined disease status.

After these, women "not fully evaluated" had at least two records (more if they also had missing VIAM or second tests results) with certain weights and with an estimated disease status: with or without disease. The final weights used to estimate measures of performance were defined as a combination of the weights used to assign different results of screening tests to women with missing data and the ones obtaining from assigning disease status, as follows:

$$w_r = \frac{\sum_{i \in E,r} w_i}{\sum_{i \in r} w_i}$$

for:

i=1 to m

r=1 to 6 strata

m=total number of pseudo-women in stratum r

$w_i = w1_{i*} w2_i$

w1: weights after estimating VIAM missing

w2: weights after estimating second stage tests missing

- $Fw_1 = W.* p_1$
- $Fw_2 = W.* p_2$
- $Fw_3 = W.* p_3$

Where:

Fw_j = weight to be used for estimates of performance, for j=1, 2, or 3.

Using these final weights, the estimated number of women having disease was then defined as:

$$Nhg = \frac{\sum_{j \in HG} Fw_j}{\sum_{j} Fwj}$$

The estimated number of women without disease was:

$N\text{-}Nhg$

Where:

N=total number of women

Definition of measures of performance

The number of True Positives for a particular test or combination of screening tests was the estimated number of women with disease who tested positive for that test or combination of tests, and the number of True Negatives was the estimated number of women without disease who tested negative for that test or combination of tests.

The sensitivity of a particular screening test or combination of several screening tests was estimated as the ratio of the True Positives over the estimated number of women with disease; the specificity as the ratio of the True Negatives over the estimated number of women without disease; and the positive predictive value as the True Positives over the total number of women testing positive for the test or combination of tests.

The screening tests or combination of screening tests, being:

(i). VIA1

(ii). VIA_M1;

(iii). LBC1 ≥ASCUS

(iv). LBC1 ≥HSIL

(v). HPV1;

And the combinations of:

(v). VIA1 and HPV1;

(vi). VIA1 and LBC1 ≥ASCUS; or,

(vii). VIA1 and LBC1 ≥HSIL.

These methods yielded unbiased point estimates but bootstrapping was needed to estimate 95% confidence intervals.

Because of the complicated algorithm; which uses multinomial regression to deal with missing data and weighted logistic regression to take account of the fact that some groups are more likely to be fully evaluated than others; it was too difficult to calculate asymptotic standard errors; therefore we used re-sampling techniques. We choose the bootstrapping approach, in which samples with replacement are drawn from the original one and the algorithm is repeated. Based on the percentile confidence interval bootstrapping estimation, after 1000 replications, 95% confidence intervals for each measure of test performance were obtained. Bootstrapping was slow but results were available sooner (Stata code for algorithm and bootstrapping on Appendix).

4.3.4. Sample size considerations

The sample size was calculated to estimate sensitivity and specificity of each test and their different combinations. Assuming three different rates of histologically confirmed high-grade disease: 1.6%, 2% and 3% in 5,000 women screened, then 80, 100 and 150 cases of high-grade disease would be expected.

Tables 4.3.4.1 and 4.3.4.2 show the power to estimate sensitivity and specificity of any screening test (or different combinations of screening tests) assuming true sensitivities vary between 65% and 90%, and true specificities between 83% and 98%. The power is obtained for a one-sided test, using the normal approximation, for an α=0.05, and for an alternative hypothesis that the true minimum sensitivity or specificity equals the different values proposed, and the difference with the null hypothesised value is not more than 10%.

Table 4.3.4.1. Power for different assumed sensitivities and rates of high-grade disease.

Null Hypothesis Ho:	Alternative Hypothesis Ha:	Expected number of women with histologically high-grade disease n=150 Power	n=100 Power	n=80 Power
Sens=0.95	Sens>0.85	99%	68%	62%
Sens=0.90	Sens>0.80	97%	90%	84%
Sens=0.85	Sens>0.75	93%	83%	76%
Sens=0.75	Sens>0.65	86%	73%	65%
Sens=0.65	Sens>0.55	81%	67%	59%

A sample size of 5,000 women will guaranteed an accurate estimation of any specificity over 78% for any screening test or combination of tests.

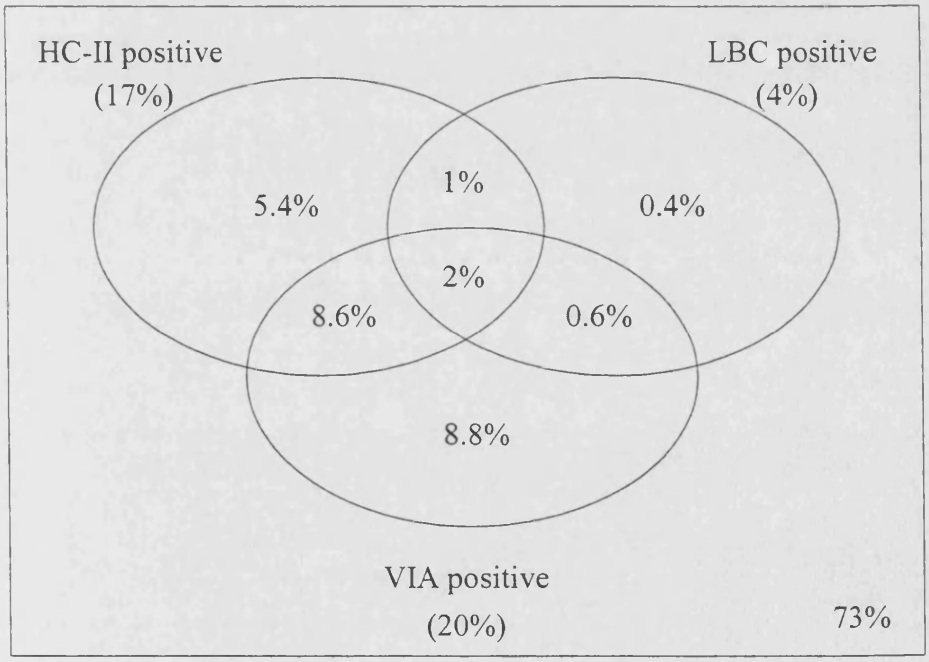
Sensitivity estimation depends on rate of disease, in the worst scenario: only 80 cases of high-grade disease are detected, there will not be enough power to detect sensitivities under 75%, and the estimates will not be very precise. But, if 150 cases are detected, the estimation of sensitivity is guaranteed; assuming sensitivities are in the range of 55% to 85%.

Table 4.3.4.2. Power for different assumed specificities and rates of high-grade disease (allowing for women lost to follow-up).

Null Hypothesis Ho:	Alternative Hypothesis Ha:	Expected number of women without high-grade disease* n=4800 Power	n=4500 Power	n=4000 Power
Spec=0.98	Spec>0.973	94%	93%	91%
Spec=0.95	Spec>0.941	87%	85%	81%
Spec=0.90	Spec>0.887	90%	88%	85%
Spec=0.85	Spec>083.5	89%	87%	84%
Spec=0.80	Spec>0.785	82%	80%	76%

* Allowing for women lost to follow-up.

Figure 4.3.4.1. Expected proportions of test results based on assumptions.



The following assumptions are used to picture the number of women testing positives in different screening tests and their combinations, as

shown in Figure 4.5.1. These assumptions are based in previous unpublished data from Peru or from the literature.

- The positivity rate of VIA is 20%.
- The positivity rate of HC-II is 17%.
- The positivity rate of LBC for detecting ASCUS or worse is 4%.

In this way, for 5,000 women screened in the San Martin region, we would expect:

- 130 women testing positive on VIA and LBC.
- 3930 women testing negative on VIA and LBC.
- 530 women testing positive on VIA and HC-II.
- 3680 women testing negative on VIA and LBC.
- 4000 women testing negative on VIA.

4.4. Ethical considerations

Informed consent was obtained in order to perform additional screening tests. Reasons for testing were clearly explained to all participating women. A special letter of consent including explanatory figures was used to explain the additional screening test to participants. The advantages of having additional samples were explained to women by midwives, who were trained to answer all sort of questions regarding any of the screening tests used in the study. When women were illiterate, they were asked to stamp their fingerprints in the informed consent letter. Cervical samples for LBC and HPV were processed nameless. Results were given to health centres, where personnel incorporated them into clinical records; which are confidential and belong to the health facility. Women were told their results in private, and if necessary, these were also explained to their partners.

4.5. My contribution

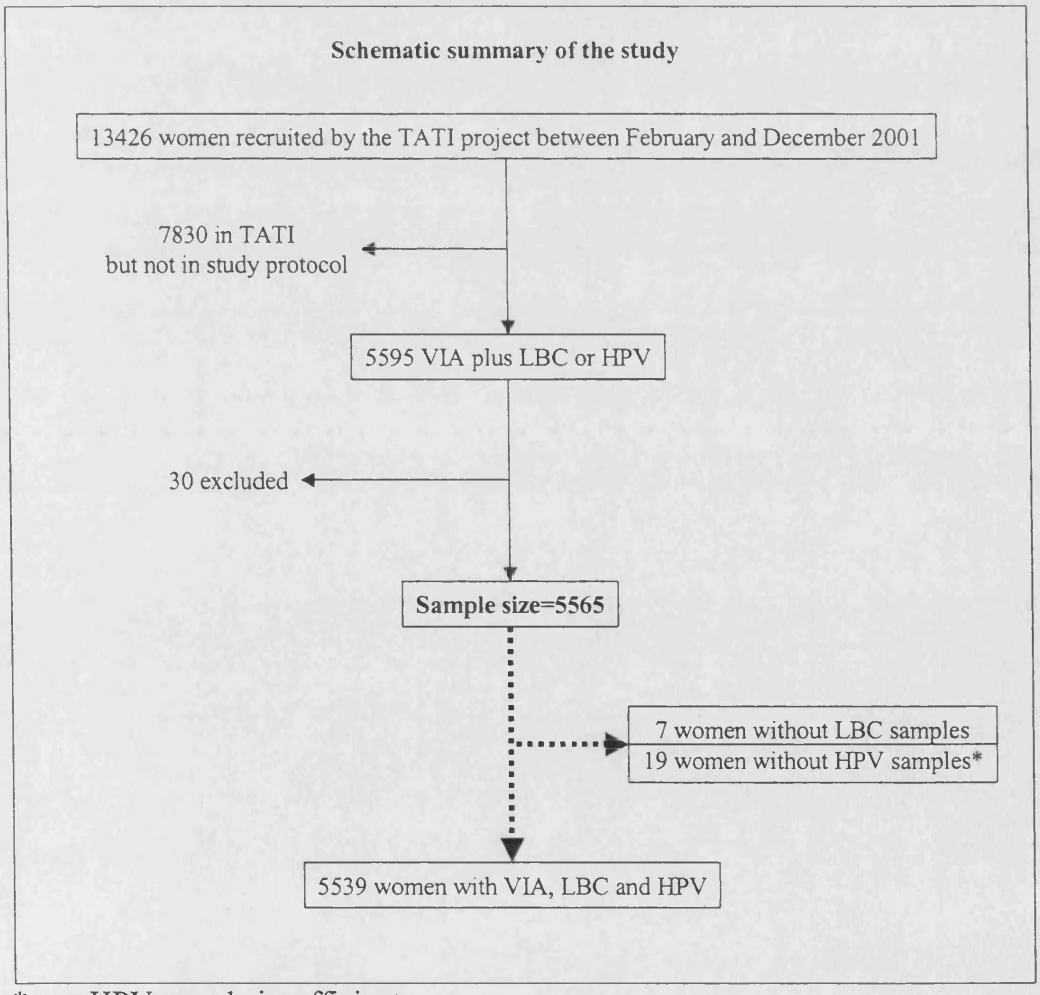
Here, I state that I developed the original protocol; which after some amendments has been used in this study. Before this study started, I collaborated intensively with the TATI investigators in the operationalization of the project. I participated in several meetings with health authorities in Lima and San Martin, while setting up the project. I helped during the first VIA and VIAM training course. I participated in the elaboration of data forms, those related to this study and to the TATI project, including the informed consent. I also collaborated with people involved in the design of the TATI database (SYSTAT). I was in charge of the implementation of the new LBC laboratory in Lima, and of organising the transport of HPV and LBC samples to Tarapoto, Lima and London; and also of the arrangement of histology being read at INEN and its quality assessment. I made regular visits to the health centres involved in my study to personally encourage midwives to continue with recruitment; and to deliver sampling kits and to collect samples. I was in charge of managing the results of LBC and HPV testing, and I made them available as soon as possible for women, and helped to identify cases missed by initial screening with VIA. I performed several quality assessments of the data of the participants of my study and showed my results to the TATI office for their use. I carried out all the analysis. Methods for dealing with missing data were proposed by my supervisor. I am the sole author of this dissertation. I would like to thank and acknowledge midwives, doctors, gynaecologists, the LBC lab, the HPV lab and the pathologists for their contribution.

5. RESULTS

5.1. Descriptive Statistics

Between February and December 2001, 5595 of 13426 women recruited by the TATI project, were also screened with LBC or HPV testing. Women were recruited from 16 health centres distributed across the region. If resources were available at the time of visit, women were offered the additional screening tests. Figure 5.1.1 shows a schematic summary of the study.

Figure 5.1.1. Schematic summary of the study



* one HPV sample insufficient.

Midwives took LBC and HPV samples on 4 women but postponed VIA for several months. Thirty women, including these 4, were excluded from analysis; the reasons are explained on Table 5.1.1.

Table 5.1.1. Women excluded from analysis.

Reasons for exclusion	Number of excluded
Evaluated in TATI course 2000	6
Samples collected after VIA positive	2
Samples collected after cryotherapy	17
VIA not performed	4
LBC inadequate and HPV missing	1
Total	**30**

Among the 5565 women considered for analysis, 7 had VIA and HPV but not LBC samples collected, 18 had VIA and LBC but not HPV samples and one HPV sample was insufficient for testing on initial screening, leaving 5539 (99.5%) with a complete set of screening tests. Only these 5539 women with complete set of screening tests were used to estimate measures of screening tests performance (see 5.5).

5.1.1. Time and place of screening

Table 5.1.1.1 shows the recruitment period and number of women recruited by each health centre. A total of 1881 (34%), 2060 (37%) and 1624 (29%) women were screened in the north, centre and south of the region, respectively.

Except for P.S. Juan Guerra and C.S. Jepelacio, each health centre recruited at least 200 women for this study. But, the Centro Materno Perinatal in Tarapoto (main city of the region), the C.S. Llluyllucucha in Moyobamba (capital of the region) and H.R. Saposoa, contributed with more than 500 women each.

Table 5.1.1.1. Number of screened women by place of screening

Health centre	Recruitment period	Screened women	
		n	%
North		1881	33.8
C.S. Lluyllucucha	Feb-Sep	586	10.5
C.S Soritor	Feb-Oct	335	6.0
C.S. Jepelacio	Jun-Aug	126	2.3
C.S. Nueva Rioja	Feb-Nov	288	5.2
H.R. Nueva Cajamarca	Feb-Nov	298	5.4
P.S. San Juan del Rio Soritor	Feb-Sep	248	4.4
Centre		2060	37.0
Centro Materno Perinatal	Apr-Aug	618	11.1
P.S. Juan Guerra	Apr-Aug	165	3.0
C.S. Tabalosos	Feb-Sep	292	5.2
H.R. Lamas	Feb-Aug	343	6.2
H.R. San Jose de Sisa	Feb-Nov	397	7.1
H.R. Picota	Feb-Aug	245	4.4
South		1624	29.2
H.R. Bellavista	Feb-Aug	349	6.3
H.R. Saposoa	Feb-Sep	556	10.0
C.S. La Merced	Feb-Aug	321	5.8
H.R. Tocache	Apr, May, Dec	398	7.1

Most health centres started recruiting patients in February and completed it by August 2001. But, the Centro Materno Perinatal and the P.S. Juan Guerra did not start until April 2001, and the C.S. Jepelacio only started in June. The HR Tocache, accessible only by small airplane, recruited women in April, May and December, when a small plane carrying sampling kits was especially flown into the city.

5.1.2. Demographics

The age distribution of 5,565 women included in the analysis is presented in Table 5.1.2.1. Despite the fact that women between 25-49 years of age were invited for screening, 61 participants were younger than 25 years of age, while 12 were older than 49 years of age. The age range was 15-56 years, and the mean (±SD) was 34.4 years (±6.5).

Table 5.1.2.1. Age distribution of screened women

	Screened women	
Age group	n	%
<25	61	1.1
25-29	1481	26.6
30-34	1480	26.6
35-39	1251	22.5
40-44	776	13.9
45-49	504	9.1
≥50	12	0.2

Table 5.1.2.2. Education level of screened women

	Screened women	
Education level	n*	%
No education	391	7.0
Incomplete primary school	1212	21.8
Complete primary school	1743	31.4
Incomplete secondary school	907	16.3
Complete secondary school	834	15.0
Higher education	471	8.5

* Data was not recorded for 7 women (n=5558).

Most screened women declared themselves as literate (93%), however, only 24% of them had completed secondary school or had higher education (Table 5.1.2.2).

The majority of women were "Mestizo", but 264 (4.7%) belonged to Peruvian ethnic groups of the region (one woman was Chayahuita, 29 were Aguarunas, and 234 Lamas-Chachapoyas).

5.1.3. Reproductive history and sexual behaviour

Table 5.1.3.1 presents the distribution of age at first intercourse and of the number of sexual partners as reported by participants during screening.

Table 5.1.3.1 Age at first sexual intercourse and number of sexual partners.

Characteristics	Screened women	
	n	%
Age at first sexual intercourse*		
<15	643	11.6
15-16	1932	34.7
17-18	1692	30.4
19-20	770	13.8
21-24	432	7.8
≥25	95	1.7
Number of sexual partners		
1	2893	52.0
2-3	1722	31.0
≥4	950	17.0

* Data was not recorded for one woman (n=5564).

The age at first sexual intercourse ranged between 8 and 33, and the mean (±SD) was 17.1 years (±2.7 years). In general, women started having sexual relationships early in life, 77% of participants started before 19 years of age. There was one woman who was sexually abused at 8 years of age and 2 others who started sexual life at 10 years of age.

More than 50% of women had only one sexual partner in life (at time of screening). There was one sexual worker identified among the participants (>90 sexual partners).

Table 5.1.3.2 summarises the self-reported reproductive history of participants including the use of contraceptive methods.

Only 2% of women were nulliparous, the majority had from 2 to 5 children, and 117 women had at least 10 children. Twenty-seven percent of women reported one abortion and another 17% more than one.

A total of 4214 (76%) women reported having ever used contraceptive methods, 58% of them specified the current contraceptive method at time of screening.

Hormonal methods were the most common (55%) including 567 women taking the pill and 761 using injectables. Sterilization had been performed in 584 (24%) women, and six husbands had a vasectomy. Six percent of participants reported using natural methods such as rhythm and breastfeeding. The duration of using the contraceptive method was also recorded but data were very inconsistent and are not presented here.

Table 5.3.1.3 summarises the previous screening history of participants as reported during screening.

Only 873 (16%) of women were previously unscreened, 28% had had cytology only once, 25% had had it twice, 18% had been cytological screened three times, and 13% had more than 3 previous cytologies.

Table 5.1.3.2 Self-reported reproductive history

Characteristics	Screened women n	%
Parity		
0	116	2.1
1	580	10.4
2	1280	23.0
3	1182	21.2
4	890	16.0
5	533	9.6
6-9	867	15.6
≥10	117	2.1
Abortions (including miscarriages)		
0	3136	56.3
1	1501	27.0
≥2	928	16.7
Ever use of Contraceptive Methods		
Yes	4214	75.7
No	1351	24.3
Current Contraceptive Method**		
None	1351	24.3
Sterilization	584	23.7
Hormonal methods	1354	55.0
IUD	172	7.0
Spermicides	30	1.2
Barrier methods	136	5.5
Natural methods	167	6.8
Other***	19	0.8

* Data on age at first intercourse was missing for one woman (n=5564)

** Percentages based on 2462 reported current contraceptive methods (Percentage of None based on 5565 women).

***Including 6 vasectomies.

Table 5.1.3.3 Self-reported screening history

Characteristics	Screened women	
	n	%
Number of previous cytologies		
0	873	15.7
1	1541	27.7
2	1408	25.3
3	978	17.6
≥4	765	13.7
Self-reported results from last cytology*		
Positive	50	1.1
Negative	3439	73.3
Does not know	1203	25.6
Self-reported year of last cytology**		
2001	406	8.8
2000	2056	44.2
1999	1000	21.5
Before 1999	1186	25.5

* Percentages based on women with data (n=4692)
** Percentages based on women with data (n=4648)

The large proportion of women previously screened is explained by the fact that the region of San Martin has been used to evaluate programmes of contraception for several years. Women seeking contraception advice were offered screening as part of the program. However, among those who had previous cytology screening, 73% reported that their last cytology was negative, only 50 recalled having received a positive result and 1204 did not know the result of their last cytology. These 50 women with a previous positive cytology were included in the analysis, since no record of their previous cytology was found in the screening centres, they were not previously treated and came asymptomatic into screening.

Only 9% of women reporting previous cytology were screened in 2001, before participating in the project. The majority of women with previous cytology were last screened in 2000 (44%), 22% were screened in 1999 and 26% before 1999.

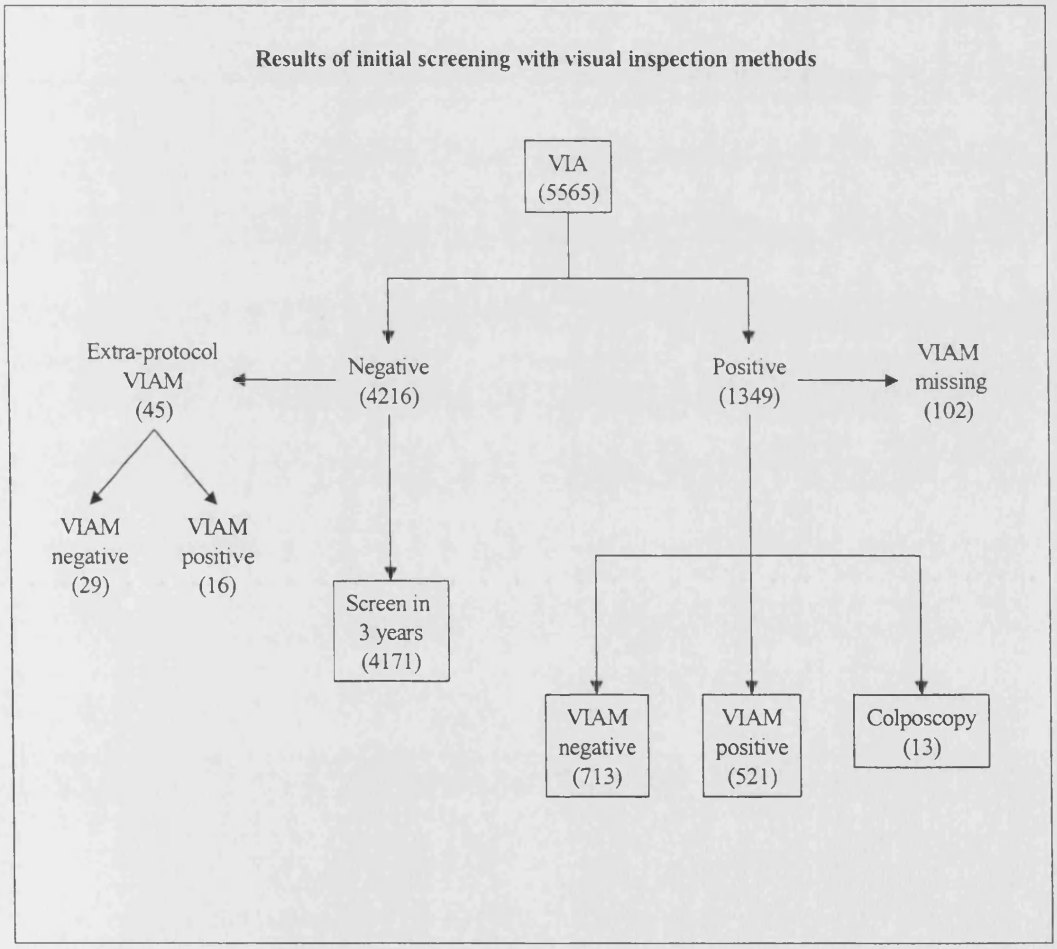
Women were also asked if they have ever smoked, and if so for how long, 97% of them had never smoked, 1.4% were current light smokers and 1.5% were ex-light smokers (data was not recorded on 4 women).

5.2. Screening tests

Of the 5565 women included in the analysis, 5539 (99.5%) had the three screening tests.

Figure 5.2.1 summarises the results of the first screening with visual inspection methods.

Figure 5.2.1. Results of initial screening with visual inspection methods.



A total of 4216 women were classified as negative with VIA. However, 45 of them were examined with VIAM outside protocol guidelines. Sixteen of them tested positive on VIAM and were treated or referred for appropriate evaluation. The remaining 4171 VIA negative women did not require further evaluation.

Among the 1349 women positive on VIA, 13 were referred directly to a colposcopist, and the rest to VIAM. Among, 1233 women who had VIAM after having a positive VIA, 521 (42%) were positive and 713 (58%) were negative. Eleven of the 521 positive VIAM women were classified as "suspected neoplasia" and were referred to colposcopy. VIAM was not performed in 102 of 1336 women who were previously referred to VIAM by the midwife performing the initial VIA.

Table 5.2.0.1 summarizes the results of initial screening with conventional cytology (CC) and liquid-based cytology (LBC).

Table 5.2.0.1. Results of Cytology: CC and LBC.

	CC		LBC	
	Screened women		Screened women	
Cytology result	n	%	n	%
Inadequate	620	11.1	301	5.4
Normal	4712	84.7	4311	77.5
ASCUS/AGUS	12	0.2	162	3.0
Condyloma/HPV	8	0.1	279	5.0
Mild dysplasia	30	0.5	334	6.0
Moderate dysplasia	25	0.5	97	1.7
Severe dysplasia/CIS	22	0.4	56	1.0
Invasive Cancer	3	0.1	18	0.3
Missing	133*	2.4	7	0.1

* Including 22 collected slides that arrived broken to the laboratory.

A total of 5454 women were screened with conventional cytology (CC), including 22 women whose samples were broken before reaching the laboratory. CC samples were inadequate in 620 (11%) women. Eighty-five

percent of women were classified as negative, only 6 ASCUS, 6 AGUS and 8 condylomas or HPV infections were reported. Only 30 mild, 25 moderate, 22 severe dysplasias and 3 suspected cancers were reported (the three of them have been histologically confirmed).

A very different picture was that of LBC. Only 5% of LBC collected samples were inadequate for laboratory analysis and 7 (0.1%) women did not have samples collected. The majority of women (4311 of 5558) tested negative on LBC. Only two samples were reported as AGUS, but 160 were considered ASCUS. Five-percent were classified as condyloma or HPV infection and 334 women had mild dysplasia and 171 had high-grade disease, including 18 suspected cancers. Of these 18 women, 14 had histologically confirmed high-grade disease or worse (1 severe dysplasia, 7 carcinomas in situ (CIS), 1 microinvasive cancer and 5 invasive cancers), one had a negative colposcopy (no biopsy was collected), and the other three have not been fully evaluated yet.

A total of 702 women (12.6%) were HPV positive on Hybrid Capture II, but 18 samples were not collected and one was insufficient for laboratory testing.

Table 5.2.0.2 presents the positivity rates of the screening tests. Two different thresholds are including for LBC: ASCUS or worse (including ASCUS, AGUS and condylomas or HPV infections, and mild or worse dysplasias) and high-grade disease or worse (including moderate dysplasias, severe dysplasias or CIS and invasive cancer). In this table, inadequate samples and missing tests are not counted.

The positivity rate of VIA alone was 24.2%, of VIAM in VIA positive women was 42.8%, and of VIA_M, i.e., combined VIA/VIAM including women referred straight to colposcopy instead of to VIAM, was 10.4%. LBC had a positivity rate of 18% for ASCUS or worse and 3% for high-grade disease, as compared to 2% positivity rate of CC for high-grade disease (as stated earlier in 4.1.3 and 4.2.2; CC positivity rates will only be tabulated for high-grade disease). The positivity rates of HPV testing

(using Hybrid Capture II) were 12.7% and 10.2% for cut-off points of 1 and 4 RLUs, respectively.

When including inadequate samples (as negative) the positivity rates of LBC for detecting low-grade disease (ASCUS or worse) was 17% and of LBC and CC for detecting high-grade disease were 3.1% and 1.8%, respectively.

Table 5.2.0.2. Positivity rates of screening tests

Screening test	Positive women n	# of adequate	Positivity Rate %
VIA	1349	5565	24.2
Combined VIA/VIAM	534	1247	10.4*
LBC ≥ ASCUS	946	5257	18.0
LBC ≥ HSIL	171	5257	3.3
CC ≥ HSIL	50	4812	1.0
HPV (≥1 RLU)	702	5546	12.7
HPV (≥4 RLU)	568	5546	10.2

* VIAM was performed in 92% of those with positive VIA. The positivity rate of Combined VIA/VIAM has been corrected by the positivity rate of VIA.

5.2.1. Screening tests and age

Table 5.2.1.1 shows the age distribution of women who tested positive on initial VIA and VIA_M.

Table 5.2.1.1. Age distribution of VIA and VIA_M positive women

	Women testing positive on VIA		Women testing positive on VIA_M	
Age-group	n	%	n	%*
<30	385	25.0	164	11.8
30-34	380	25.7	156	11.1
35-39	318	25.4	118	10.0
40-44	168	21.7	60	8.8
>44	98	19.0	36	7.4
Total	1349	24.2	534	10.4

*Positivity rate weighted by the one of VIA alone.

The positivity rate of VIA varied according to the age of screened women. It increased slowly from 25%, in those less than 30 years of age, up to 26% in those aged 31-34, but afterwards shows a decreasing trend up to 19% in those older than 44 years of age (p-value for trend=0.004). There was a clear downwards trend of VIA_M positivity with age, the older the women the smaller the positivity rate of VIA_M, it was 11.8% in those less than 30 years of age and 7.4% in those older than 44 years (Wald test=-3.21, p-value=0.001).

Table 5.2.1.2 shows the age distribution of women who tested positive on LBC (low and high-grade disease thresholds). In both cases, there is a clear increasing trend of the positivity rate of LBC across age groups.

Table 5.2.1.2. Age distribution of LBC positive women

	Women testing positive on LBC			
	Low-grade disease		High-grade disease	
Age-group	n	%	n	%
<30	238	16.1	20	1.4
30-34	234	16.8	46	3.3
35-39	235	20.1	47	4.0
40-44	135	18.5	33	4.5
>44	104	21.8	25	5.2
Total	946	18.0	171	3.3

When considering the low-grade disease threshold, the positivity rate of LBC increased from 16% in women younger than 30 years of age to 22% in women older than 44 years of age (p-value for trend=0.0015). This is a surprising result, as the number of low-grade cytological abnormalities usually decreases after 40 years of age.

When considering the high-grade disease threshold, the positivity rate of LBC increased from 1.4% in those younger than 30 to 5.2% in those older than 44 years of age (p-value for trend<0.001).

Table 5.2.1.3. Age distribution of HPV positive women

	Women testing positive on HC-II	
Age-group	n	%
<30	236	15.4
30-34	183	12.4
35-39	148	11.9
40-44	89	11.5
>44	46	9.0
Total	702	12.7

Table 5.2.1.3 shows the age distribution of women who tested positive on HPV. There is a decreasing trend of the prevalence of HPV infection, as women grow older. The positivity rate of HC-II decreases from 15.4% in women younger than 30 years to 9% on women older than 44 years (p-value for trend=0.0001). Among women screened outside the established age range: three aged 15-19 years, 58 aged 20-24 and 12 aged 50-56, 1 (33.3%), 8 (13.8%) and 2 (16.7%) had a positive HPV test.

5.2.2. Screening tests and place of screening

Table 5.2.2.1 shows the distribution of place of screening among those women testing positive on VIA and on VIA_M.

Table 5.2.2.1. Place of screening of VIA and VIA_M positive women

Health centre	Women testing positive on VIA		Women testing positive on VIA_M	
	n	%	n	%*
North	441	23.4	180	11.3
C.S. Lluyllucucha	141	24.1	55	10.3
C.S Soritor	33	9.9	11	4.9
C.S. Jepelacio	70	55.6	6	6.0
C.S. Nueva Rioja	37	12.9	22	9.7
H.R. Nueva Cajamarca	95	31.9	48	19.1
P.S. San Juan del Rio Soritor	65	26.2	38	17.5
Centre	671	32.6	250	12.6
Centro Materno Perinatal	266	43.0	73	12.0
P.S. Juan Guerra	54	32.7	14	9.8
C.S. Tabalosos	77	26.4	33	11.6
H.R. Lamas	130	37.9	47	14.1
H.R. San Jose de Sisa	115	29.0	71	18.5
H.R. Picota	29	11.8	12	5.9
South	237	14.6	104	6.6
H.R. Bellavista	98	28.1	18	5.3
H.R. Saposoa	40	7.2	19	3.4
C.S. La Merced	26	8.1	25	8.1
H.R. Tocache	73	18.3	42	11.0
Total	1349	100	534	100

*Positivity rate weighted by the one of VIA alone.

The positivity rate of VIA varied greatly (Chi-square=465.8,df=15,p-value<0.0001), from 7.2% in women screened at H.R. Saposoa (in the south) to 55.6% in women screened at C.S. Jepelacio (in the north).

On average, VIA positivity rates were higher in the centre of the region (32.6%), with Centro Materno Perinatal having the highest rate (43% of more than 600 screened women), while the lowest rates came from the south (14.6%).

The positivity rates of VIA_M also varied across health centres, but more moderately than those of VIA (Wald test=0.12,p-value=0.908). VIA_M positivity rates range was 3.4-19% (in H.R. Saposoa and H.R. Nueva Cajamarca, respectively).

VIA_M positivity rates were higher in the centre of the region (12.6%) and lower in the south (6.6%), (Wald test=-1.34,p-value=0.181).

Figure 5.2.2.1. Positivity rates of VIA_M and VIA in 16 health centres.

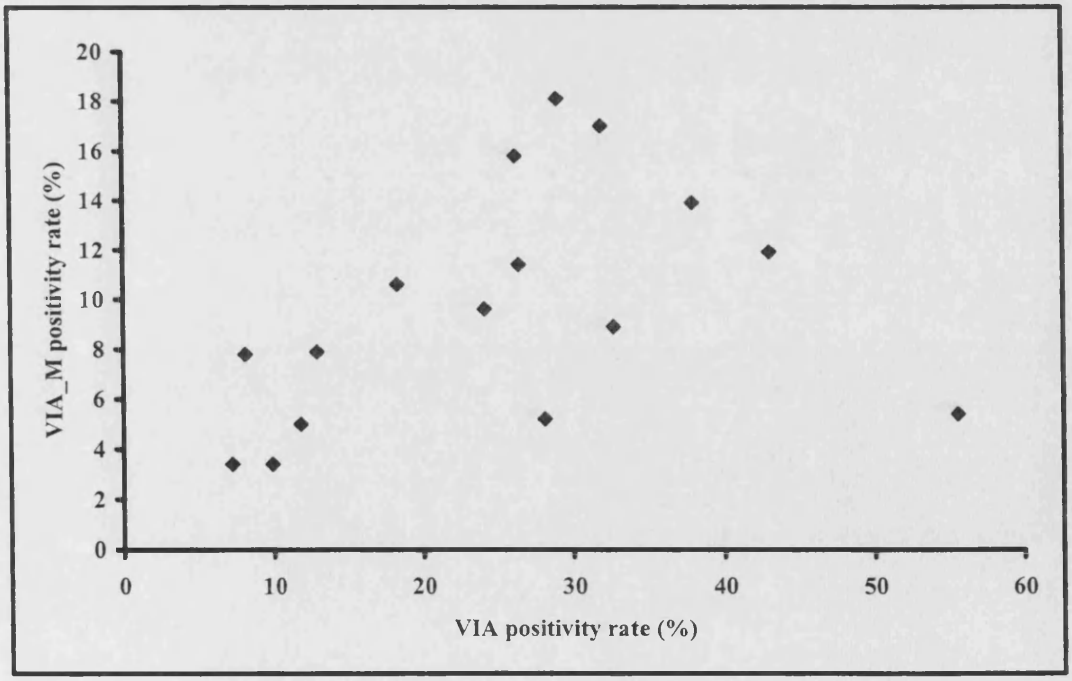


Figure 5.2.2.1 presents the positivity rates of VIA and VIA_M by health centre. This figure suggests that the positivity rates of both screening tests

are weakly correlated. If the variability of VIA positivity rates was due to variability of disease in different health centres, this figure should show a significant correlation between both tests positivity rates. But it seems that among health centres with VIA positivity rates under 40%, there was a positive correlation between midwives' and doctors' diagnosis while the contrary occurred in health centres with VIA positivity rates over 40%. This suggests that much of the variability of VIA positivity rates is due to differences in VIA interpretation rather than differences in the underlying rates of visible lesions.

Table 5.2.2.2. LBC and CC inadequate samples by place of screening.

Health centre	Inadequate samples			
	LBC		CC	
	n	%	n	%
C.S. Lluyllucucha	30	10.0	33	5.3
C.S Soritor	5	1.7	9	1.5
C.S. Jepelacio	6	2.0	13	2.1
C.S. Nueva Rioja	9	3.0	89	14.4
H.R. Nueva Cajamarca	23	7.6	32	5.2
P.S. San Juan del Rio Soritor	9	3.0	75	12.1
Centro Materno Perinatal	40	13.4	48	7.7
P.S. Juan Guerra	17	5.6	28	4.5
C.S. Tabalosos	17	5.6	59	9.5
H.R. Lamas	20	6.6	69	11.1
H.R. San Jose de Sisa	13	4.3	31	5.0
H.R. Picota	25	8.3	52	8.4
H.R. Bellavista	34	11.3	55	8.9
H.R. Saposoa	19	6.3	12	1.9
C.S. La Merced	24	8.0	3	0.5
H.R. Tocache	10	3.3	12	1.9
Total	301	100.0	620	100.0

Table 5.2.2.2 shows the distribution of LBC and CC inadequate samples by place of screening. The number of inadequate samples varied from 1.7 to 13.4 for LBC and between 0.5 and 14.4 for CC. The percentage of inadequate samples was roughly similar for both LBC and CC in C.S. Soritor, C.S. Jepelacio, P.S. Juan Guerra, H.R. San Jose de Sisa and H.R. Picota, larger for LBC than CC in C.S. Lluyllucucha, H.R. Nueva Cajamarca, Centro Materno Perinatal, H.R. Bellavista, H.R. Saposoa, C.S. La Merced and H.R. Tocache, and smaller for LBC than CC in the rest.

Table 5.2.2.3 shows the distribution of place of screening among those women with low-grade or high-grade disease on LBC.

When considering the low-grade disease threshold, the positivity rate of LBC varied from 12.8% in the Centro Materno Perinatal (centre of the region) to 29.4% (Chi-square, df=15, p-value<0.0001) in the P.S. San Juan del Rio Soritor (north).

On average, health centres in the north of the region had the highest LBC (low-grade) positivity rate (21.1%) while those in the centre of the region had the lowest (15%).

When a high-grade disease threshold was considered, the positivity rates of LBC varied from 1.9% in the H.R. Lamas (centre of the region) to 5.0% in the P.S. San Juan del Rio Soritor (north). This variation was not statistically significant (Chi-square, df=15, p-value=0.771).

Overall, the positivity rates of LBC for detecting high-grade disease were 2.5% in the centre of the region, 3.6% in the north and 3.7% in the south.

LBC positivity rates of detecting ASCUS or worse were more steady than those of VIA across health centres; but distribution of rates was different. For LBC (≥ASCUS), the positivity rates were higher in the north of the region (21%) while for VIA, they were higher in the centre (33%).

Table 5.2.2.3. Place of screening of LBC positive women

Health centre	Women testing positive on LBC			
	Low-grade disease		High-grade disease	
	n	%	n	%
North	378	21.1	65	3.6
C.S. Lluyllucucha	94	17.0	15	2.7
C.S Soritor	79	23.9	13	3.9
C.S. Jepelacio	20	16.7	3	2.5
C.S. Nueva Rioja	52	18.6	10	3.6
H.R. Nueva Cajamarca	63	23.0	12	4.4
P.S. San Juan del Rio Soritor	70	29.4	12	5.0
Centre	289	15.0	49	2.5
Centro Materno Perinatal	74	12.8	13	2.3
P.S. Juan Guerra	21	14.2	4	2.7
C.S. Tabalosos	41	14.9	8	2.9
H.R. Lamas	53	16.4	6	1.9
H.R. San Jose de Sisa	66	17.2	13	3.4
H.R. Picota	34	15.5	5	2.3
South	279	18.2	57	3.7
H.R. Bellavista	62	19.7	12	3.8
H.R. Saposoa	96	17.9	19	3.5
C.S. La Merced	42	14.1	11	3.7
H.R. Tocache	79	20.4	15	3.9

Figure 5.2.2.2 and 5.2.23 show the positivity rates LBC (ASCUS+) and LBC (HSIL+) positivity rates by health centre. In both cases, the positivity rates of LBC and VIA_M are poorly correlated.

Figure 5.2.2.2. Positivity rates of LBC (ASCUS+) and VIA_M in 16 health centres.

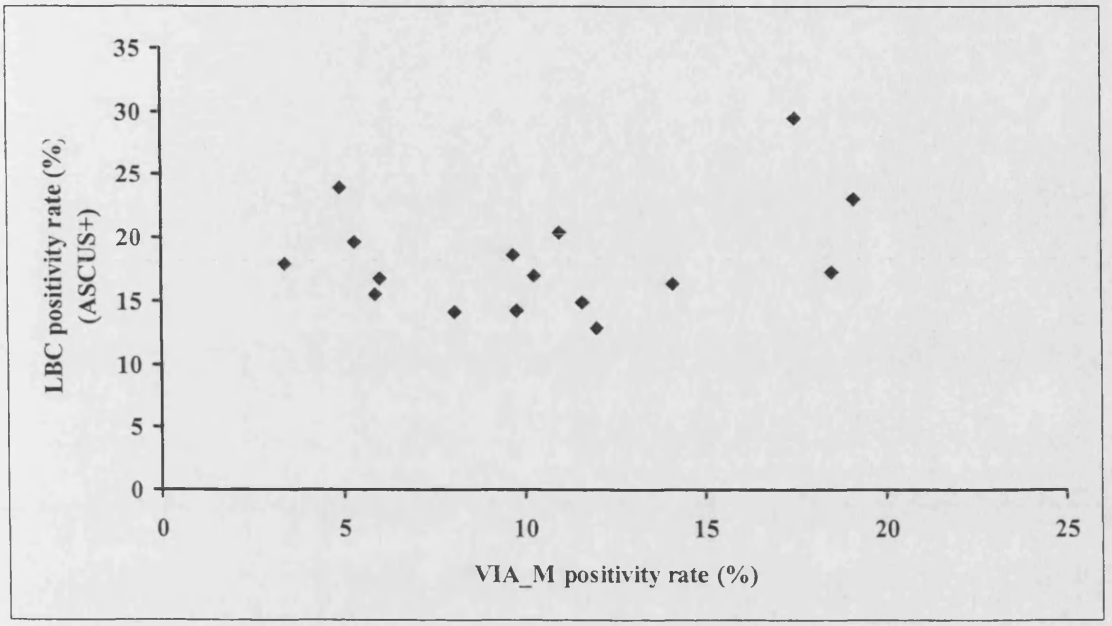


Figure 5.2.2.3. Positivity rates of LBC (HSIL+) and VIA_M in 16 health centres.

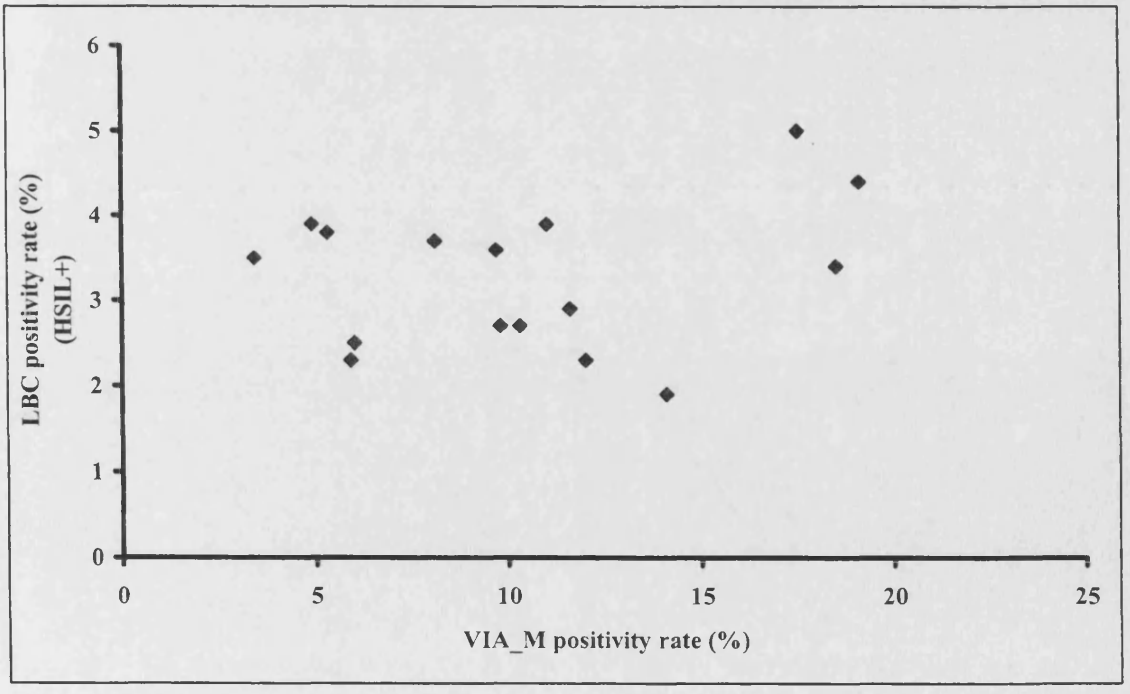


Table 5.2.2.4 shows the distribution of place of screening among those women testing positive on HC-II.

Table 5.2.2.4. Place of screening of HPV positive women

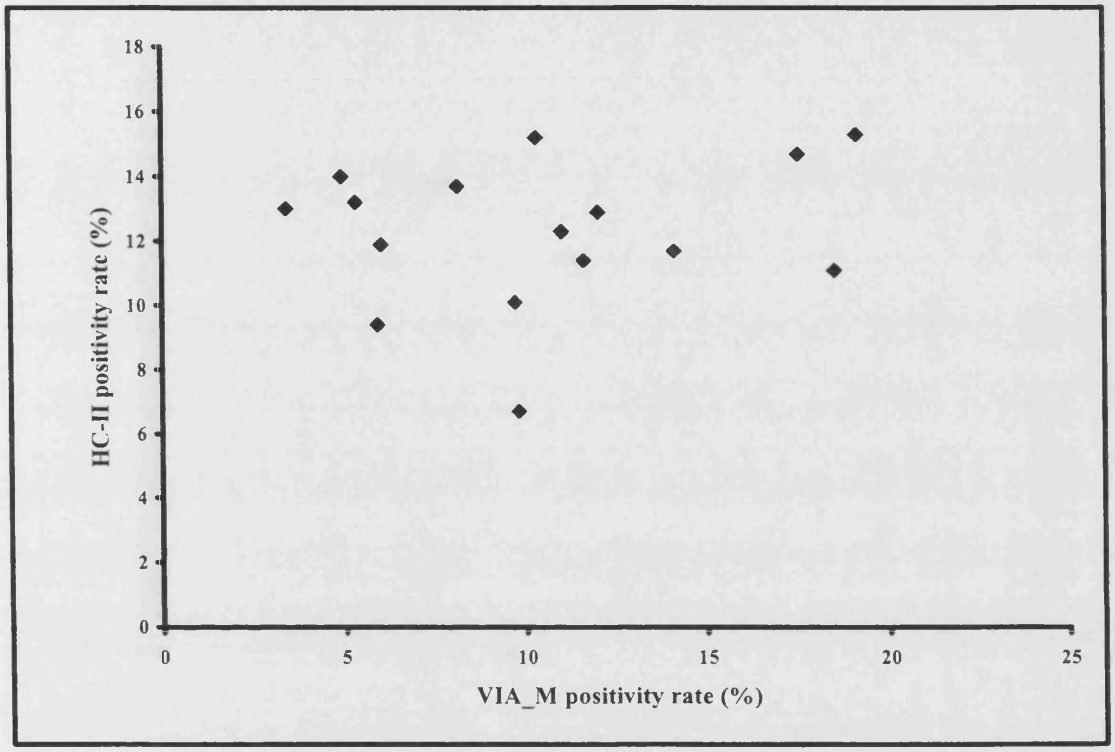
Health centre	Women testing positive on HC-II	
	n	%
North	260	13.9
C.S. Lluyllucucha	88	15.2
C.S Soritor	47	14.0
C.S. Jepelacio	15	11.9
C.S. Nueva Rioja	29	10.1
H.R. Nueva Cajamarca	45	15.3
P.S. San Juan del Rio Soritor	36	14.7
Centre	231	11.2
Centro Materno Perinatal	80	12.9
P.S. Juan Guerra	11	6.7
C.S. Tabalosos	33	11.4
H.R. Lamas	40	11.7
H.R. San Jose de Sisa	44	11.1
H.R. Picota	23	9.4
South	211	13.0
H.R. Bellavista	46	13.2
H.R. Saposoa	72	13.0
C.S. La Merced	44	13.7
H.R. Tocache	49	12.3

The positivity rate of HPV testing varied from 6.7% in the P.S. Juan Guerra (centre of the region) to 15.3% in the H.R. Nueva Cajamarca (in the north). However, most positivity rates were between 11% and 13%, making the variation not statistically significant (Chi-square=18.3,df=15, p-value=0.249).

Figure 5.2.2.4 shows the positivity rates of HC-II and VIA_M in the 16 health centres participating in the study. Overall, the HC_II positivity rates

do not vary as VIA_M positivity rates increase, and their correlation is very poor.

Figure 5.2.2.4. Positivity rates of HC-II and VIA_M in 16 health centres.



5.2.3. Combination of screening tests

Tables 5.2.3.1 and 5.2.3.2 present the detailed results of LBC and HPV by VIA, these results are summarised in Table 5.2.3.3 in which the positivity rates of VIAM, LBC, CC and HPV for different thresholds are presented.

Table 5.2.3.1 shows the results of LBC according to VIA. Among 1349 women who tested positive on VIA, 78 (5.8%) LBC samples were inadequate, 999 (74.1%) were LBC negative, 3% had ASCUS, 4.7% had condyloma or HPV infection, 7% had mild dysplasia, and 74 (5.4%) had HSIL including 11 suspected cancers.

Table 5.2.3.1. LBC results by VIA.

	VIA Positive		VIA Negative	
Liquid-Based Cytology result	n	%	n	%
Inadequate	78	5.8	223	5.3
Negative	999	74.1	3212	78.6
ASCUS	41	3.0	121	2.9
Condyloma/HPV	64	4.7	215	5.1
Mild dysplasia	93	7.0	241	5.7
Moderate dysplasia	45	3.3	52	1.2
Severe dysplasia/CIS	18	1.3	38	0.9
Invasive cancer	11	0.8	7	0.2*

* Seven women negative on VIA did not have LBC samples.

Conventional cytology samples among women testing positive on VIA were inadequate in 174 women and missing in 36 (5 slides were broken before arriving to the laboratory), but among 1139 adequate samples, 26 (2.3%) were reported as high-grade disease, including two suspected cancers.

Table 5.2.3.2 shows the HPV testing results according VIA. Among 1349 women testing positive on VIA, 232 (17.2%) were HPV positive, but in four of them HPV samples were not collected.

Table 5.2.3.2. HPV results on VIA positive women.

	VIA Positive		VIA Negative	
Hybrid Capture-II results	n	%	n	%
Negative	1113	82.5	3731	88.5
Positive (≥1 RLU)	232	17.2*	470	11.5**

* Four women positive on VIA did not have HPV samples.
** Fifteen women positive on VIA did not have HPV samples.

Table 5.2.3.3 summarises the positivity rates of other screening tests among VIA positive women (inadequate and missing samples are not counted on this table).

Table 5.2.3.3. Positivity rates of VIA on women positive on other screening tests.

	Women testing positive on VIA	
Screening tests	n	%
VIAM	534	42.8
LBC ≥ ASCUS	272	21.4
LBC ≥ HSIL	74	5.8
CC ≥ HSIL	26	2.3
HPV (≥1 RLU)	232	17.2
HPV (≥4 RLU)	199	14.8

As presented before in Figure 5.2.1, 1349 women tested positive on VIA and were referred to VIAM, but 102 women have not yet been examined.

Among 1247 VIA positive women who had VIAM, 534 (42.8%) tested positive on VIAM.

Among 1271 VIA positive women with adequate samples on LBC, 272 (21.4%) had ASCUS or worse, and 74 (5.8%) had high-grade disease or worse. Of 1139 women with adequate samples on CC and who tested positive on VIA, 26 (2.3%) had HSIL or worse on CC.

Table 5.2.3.4 presents the overall positivity rates of screening tests when combined with VIA. The combined test is considered positive if the second test is positive after VIA was positive and the combination is negative otherwise, excluding the inadequate and missing samples. The positivity rates presented in this table are weighted ones, because not every woman who was positive on VIA had VIAM, or had adequate cytology or HPV samples.

Table 5.2.3.4. Overall positivity rates of screening tests among women testing positive on VIA.

Screening tests	Number positive among VIA positive women	Overall proportion positive on both tests
VIAM	534	10.4
LBC ≥ ASCUS	272	5.2
LBC ≥ HSIL	74	1.4
CC ≥ HSIL	26	0.6
HPV (≥1 RLU)	232	4.2
HPV (≥4 RLU)	199	3.6

Among 1247 women who had VIAM because of being positive on VIA, 534 were positive, giving a positivity rate of combined VIA/VIAM (VIA_M) of 10.4%. Of 1271 women testing positive on VIA with adequate LBC samples, 272 had at least ASCUS and 74 had HSIL or worse, yielding combined VIA/LBC positivity rates of 5.2% and 1.4%,

respectively. Only 26 of 1139 VIA positive women with adequate CC samples had HSIL on CC with a positivity rate of 0.6%. As for HPV testing, among 1345 women testing positive on VIA with adequate HPV samples, 232 were positive on HC-II (≥1 RLU) and 199 for a cut-off point of 4RLU, yielding positivity rates of combined VIA/HPV testing of 4.2% and 3.6%, respectively.

Table 5.2.3.5 shows the number of women testing positive on different screening tests according to VIA and VIAM.

Table 5.2.3.5. Positivity rates of different screening tests according to VIA and VIAM results.

Screening test	VIA negative* n (%)	VIA positive VIAM negative** n (%)	VIA positive VIAM positive*** n (%)
LBC ≥ ASCUS	674 (16.9)	119 (18)	136 (26.7)
LBC ≥ HSIL	97 (2.4)	24 (3.6)	46 (9.0)
CC ≥ HSIL	24 (0.7)	5 (0.8)	18 (3.9)
HPV (≥1 RLU)	470 (11.2)	100 (14.0)	119 (22.5)
HPV (≥4 RLU)	369 (8.8)	83 (11.6)	104 (19.6)

* VIA negative = VIA neg in VIA_MM1 (see 4.3.2.d).
** VIA positive and VIAM negative = VIAM negative on VIA_MM1.
*** VIA positive and VIAM positive = VIA_MM1 Cryo or Colp.

Among women who were negative on VIA, 674 (16.9%) had ASCUS or worse lesions on LBC, 97 and 24 had high-grade disease on LBC and CC, respectively; 470 were positive on HC-II (≥1 RLU) and 369 were positive for a cut-off point of 4 RLUs.

Among those who were positive on VIA but negative on VIAM, 119 (18%) had at least ASCUS on LBC, 3.6% and 0.8% had HSIL on LBC and

CC, respectively; 14% and 11.6% were positive on HPV testing for cut-off points of 1 and 4 RLUs, respectively.

Among those women who tested positive in both VIA and VIAM, VIA_M positive, 26.7% had at least ASCUS on LBC, 9% and 3.9% had HSIL or worse on LBC and CC, respectively; 22.5% and 19.6% were positive on HC-II for cut-off points of 1 and 4 RLUs.

The positivity of each test increased across VIA and VIAM results, and decreased when using higher threshold definitions for cytology (ASCUS vs HSIL) and for HPV testing (1 vs 4 RLUs).

5.2.4. Second screening tests results

Among 646 women eligible for second screening, 477 had second VIA_M results, 591 had second LBC results and 563 had second HPV results available at time of analysis.

Table 5.2.4.1. Second screening test results.

	Number of women			
Second screening test	**Negative**	**%**	**Positive**	**%**
VIA_M2	448	94%	29	6%
LBC2 ≥HSIL	523	88%	68	12%
HPV2	418	75%	145	25%

Of the 29 being positive on second VIA_M, 12 had cryotherapy and 17 were referred to colposcopy. Among 523 women "negative" (no worse than LSIL) for LBC2, 12 had inadequate LBC samples, 261 were negative, 33 had ASCUS and 8 had AGUS, 130 had condyloma/HPV and 79 had mild dysplasia. Of those 68 positive, 7 women had suspected carcinoma and two had suspected adenocarcinoma.

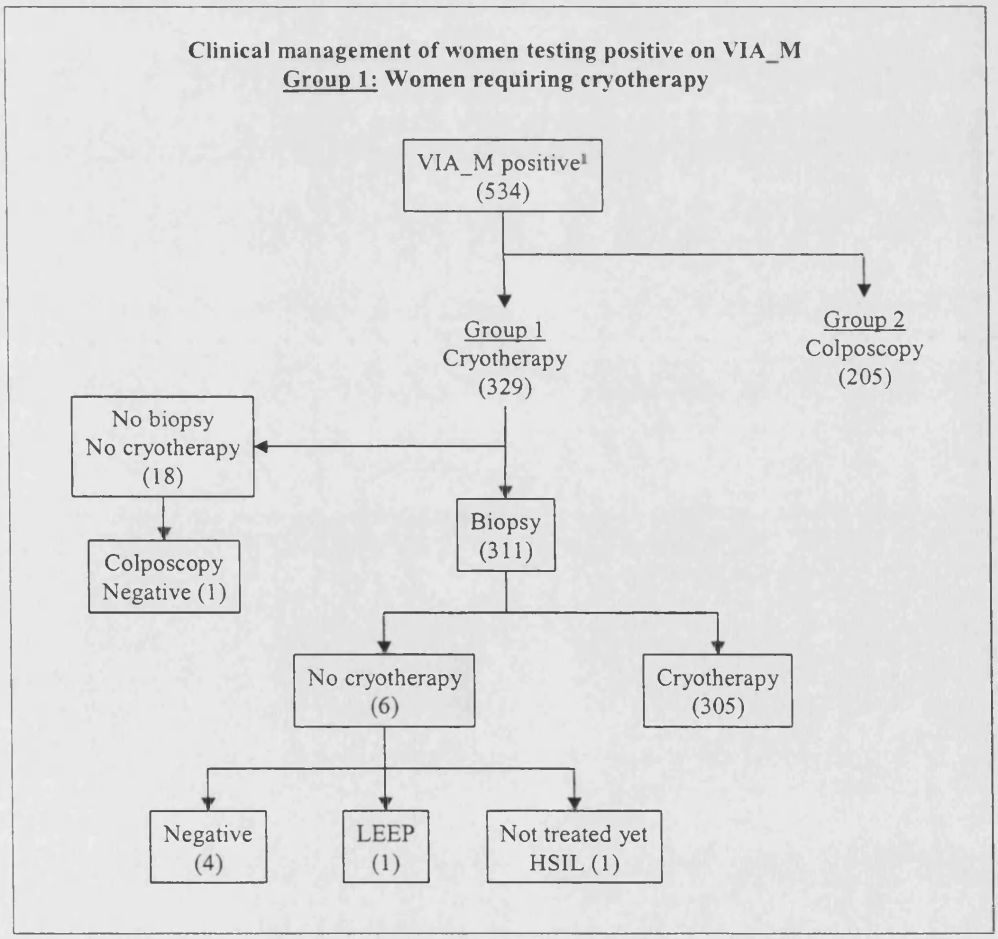
5.3. Clinical management according to screening results

The number of screened women evaluated and treated is presented following the clinical management groups established in Figure 4.1.3.2. These figures are based on original data before any estimation of missing values.

Group 1: Women requiring cryotherapy after testing positive on first VIA_M (VIA_M1).

Figure 5.3.1 presents the clinical management of women testing positive on VIA_M who required cryotherapy.

Figure 5.3.1. Clinical management of women testing positive on VIA_M who required cryotherapy.



1 Including 13 women referred straight to colposcopy after VIA.

A total of 534 women were considered positive on VIA_M1. These included 13 women referred to colposcopy by the midwife performing VIA, and 521 testing positive on VIAM.

Doctors performing VIAM indicated treatment with cryotherapy in 329 women, but in 18 cases, the treatment was postponed. There were two main reasons for postponement: women preferred to consult with their partners first, or the cryotherapy pistol had technical problems. One of these 18 women was referred to colposcopy that turned out to be negative and so no biopsy sample was collected. In other six cases, women decided to have a biopsy first and wait for the result before being treated. Four of these six women had a negative result on pathology, the other two had HSIL but only one of them has already been treated with LEEP. The remaining 305 women signed an informed consent for biopsy and cryotherapy, and were treated.

Group 2: Women referred to colposcopy after testing positive on initial VIA_M (VIA_M1).

Figure 5.3.2 presents the clinical management of women testing positive on VIA_M1 who were referred to colposcopy.

Doctors decided that cryotherapy was not appropriate for 205 women who were referred to colposcopy, 8 of these women have not been evaluated yet.

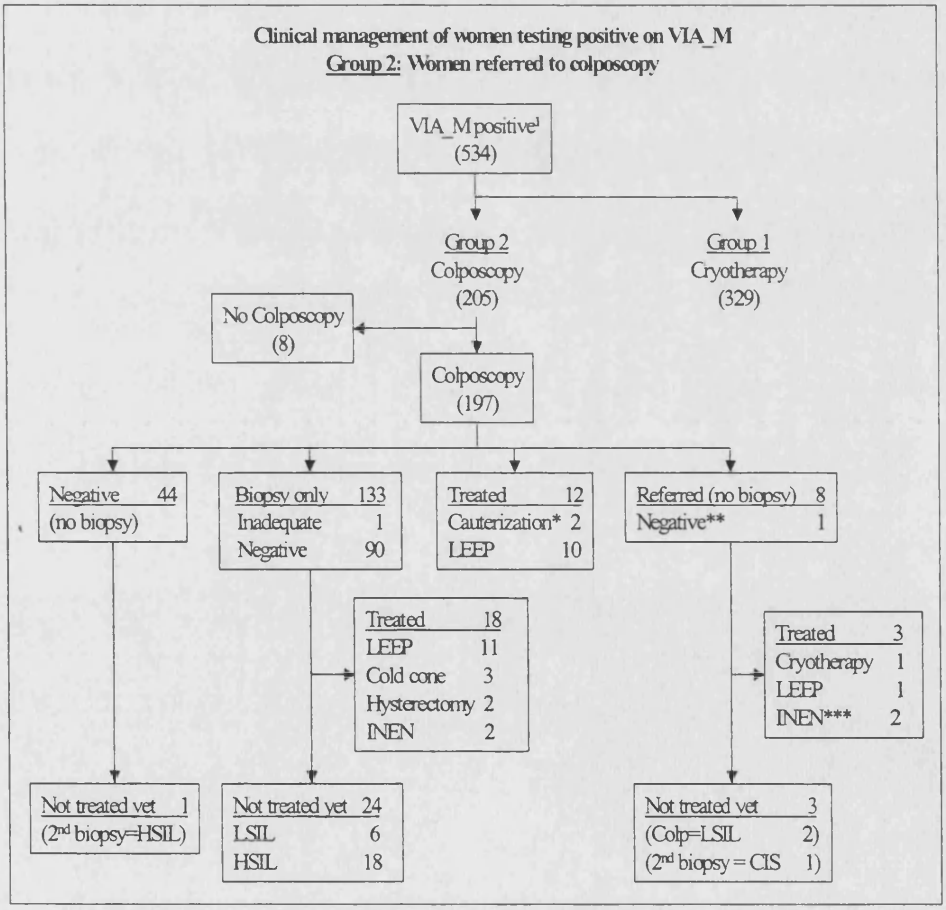
Of 197 women evaluated with colposcopy, 52 were not biopsied, 133 had a biopsy, 2 had a biopsy and were treated with cauterisation and 10 were treated with LEEP.

Among those women with no biopsy on the colposcopy, 44 were not biopsied because they were classified as negative by the colposcopist, 3 were classified as mild dysplasia and 5 as HSIL or worse. One of the women with mild dysplasia on colposcopy was referred back and treated with cryotherapy by a general doctor, and the other two have not been treated yet. One of the women with HSIL was treated with LEEP, one is

waiting to be treated, another one had a negative biopsy (on a second colposcopy), and two with suspected invasive cancer were referred to the cancer hospital in Lima (INEN).

Sixteen women who had only a biopsy on the first colposcopy were treated on a second or third visit (11 LEEPs, 3 cold conizations and 2 hysterectomies), and two women with histologically confirmed carcinomas were referred to INEN but one of them rejected treatment. Twenty-four women with histologically confirmed mild dysplasia (6) or a higher cervical lesion are still waiting to be treated.

Figure 5.3.2. Clinical management of women testing positive on initial VIA_M who were referred to colposcopy.



1 Including 13 women referred straight to colposcopy after VIA.

* Cauterization after biopsy.

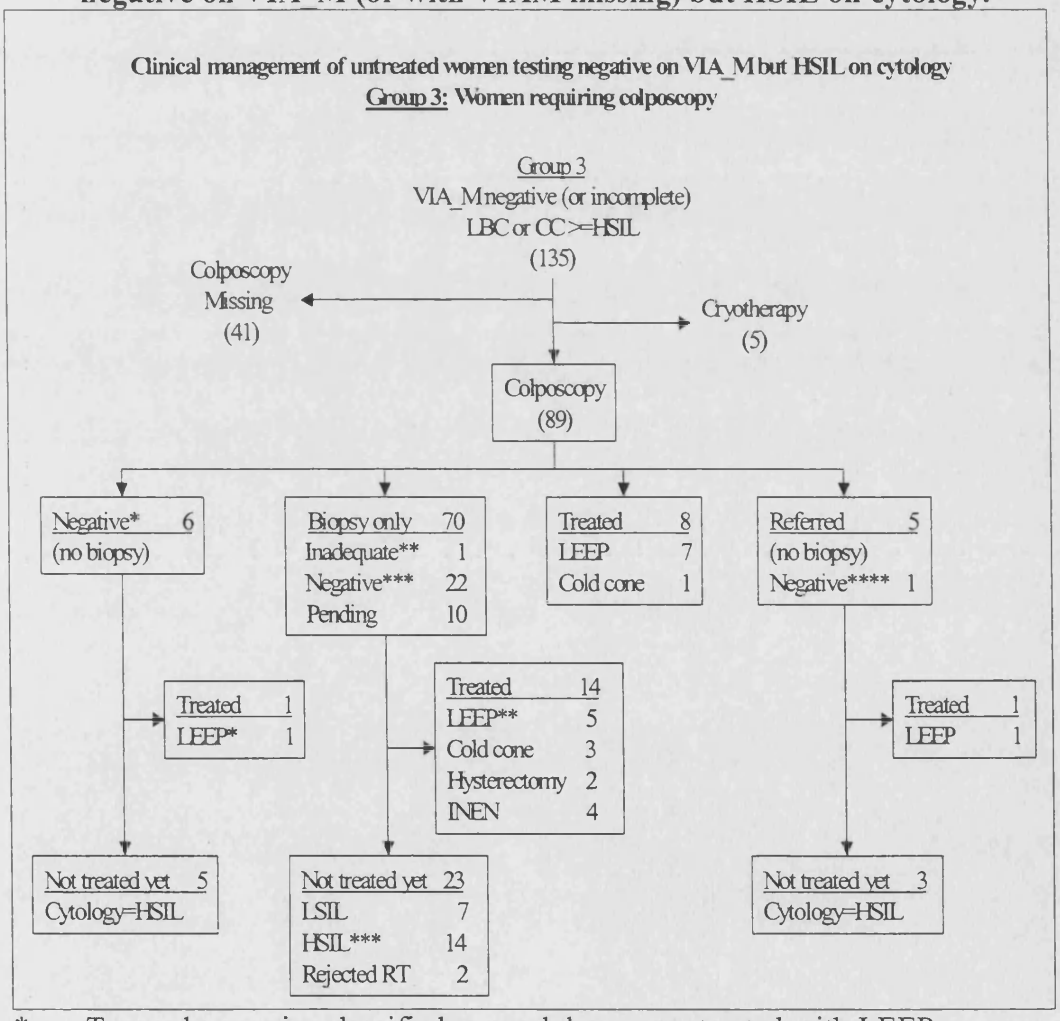
** Negative biopsy on a second colposcopy.

*** One rejected treatment at INEN.

Group 3: Untreated women requiring colposcopy after testing negative on VIA_M (or with VIAM missing) and whose cytology (LBC or CC) results were high-grade disease or worse.

A total of 135 untreated women who tested negative on VIA_M had HSIL on cytology (LBC or CC) and required colposcopy. Of them, 41 have not been colposcopically examined yet, 5 have had VIAM and cryotherapy instead, and 89 have had colposcopy.

Figure 5.3.3. Clinical management of untreated women testing negative on VIA_M (or with VIAM missing) but HSIL on cytology.



* Two colposcopies classified as condyloma, one treated with LEEP.
** One severe dysplasia on second biopsy treated with LEEP.
*** One cancer and one moderate dysplasia on second biopsies not treated yet.
**** One metaplasia on second biopsy.

Colposcopy was negative on six women including two classified as condyloma, which were not biopsied. But one of them was treated with LEEP on a second colposcopy.

Seventy women were only biopsied during first colposcopy; 10 of them were then treated (5 LEEPs, 3 cold cones, 2 hysterectomies) and 6 with suspected cancer have been referred to INEN (but two rejected treatment); two biopsies were insufficient, one of them was repeated and turned out to be a severe dysplasia that was treated with LEEP, the other has not been repeated yet. Twenty-four biopsies were negative, two of them were repeated, one resulted carcinoma and the other moderate dysplasia, and none of them have been treated yet. The other 21 biopsies were at least LSIL but have not been treated yet, and 10 pathology results are not available yet.

Eight women were treated immediately after colposcopy, seven had LEEPs and the other one had cold conization.

Another five women were diagnosed as HSIL on colposcopy but were not biopsied, one of them was treated with LEEP in a second colposcopy, 3 have not been treated yet, and one of them had a second colposcopy with a negative biopsy.

Altogether, 74 have been properly evaluated and 31 (42% of those evaluated) had HSIL on histology.

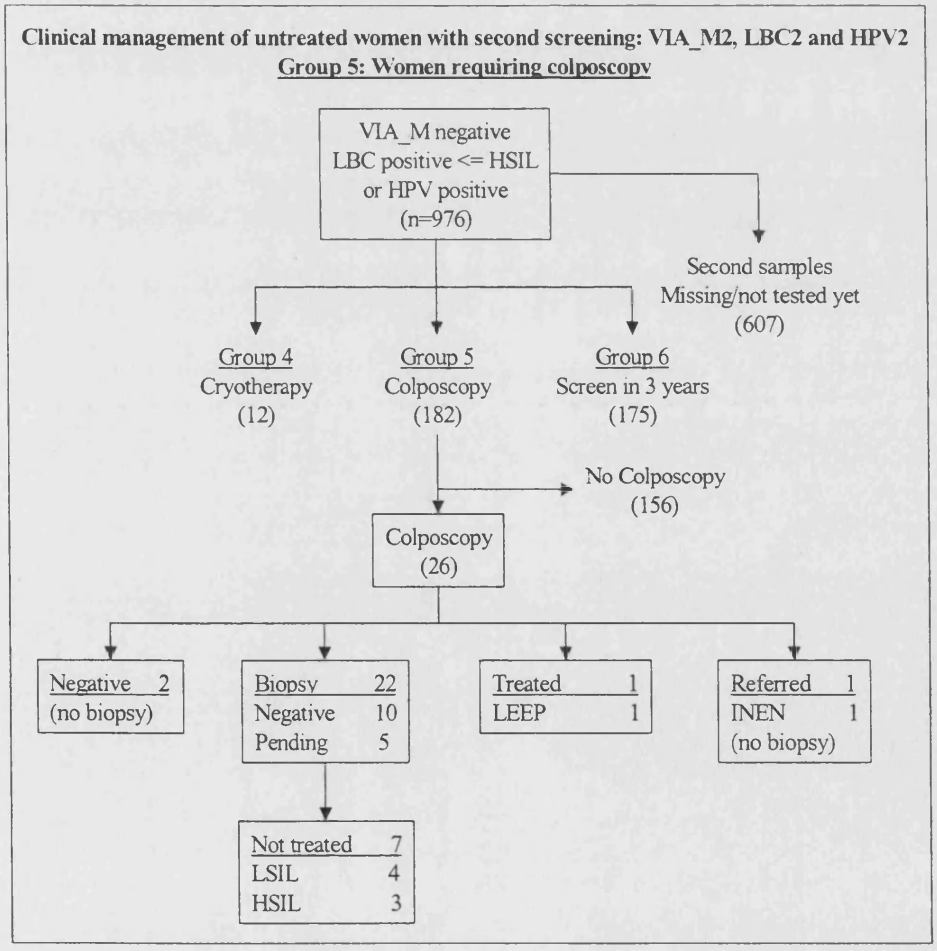
Group 4: Untreated VIA_M1 negative with second screening who required cryotherapy after testing positive on second VIA_M (VIA_M2).

There were 976 untreated women who were negative on first VIA_M but had either ASCUS/LSIL (including ASCUS, AGUS, condyloma/HPV and mild dysplasia) on LBC or tested positive on HPV. So far, 12 women have been positive on VIA_M2 and have been treated with cryotherapy. Three had mild dysplasia, two had moderate dysplasia and one had severe dysplasia on histology (biopsy taken before cryotherapy), one sample was insufficient and the other five were negative.

Group 5: Untreated VIA_M negative women with second screening, who required colposcopy (VIA_M2 positive or HSIL on second LBC or second HC-II positive).

So far, 182 women required colposcopy because of being positive on second screening: 17 because of VIA_M2, 31 because of having HSIL on second LBC, 103 because of being positive on second HPV, and 31 because of having HSIL on second LBC and being positive on second HPV.

Figure 5.3.4. Clinical management of untreated women with second screening positive (HSIL on second LBC or second HPV positive).



Among 26 women who have had colposcopy, two were classified as negative and no biopsy was collected, 22 were only biopsied, one was

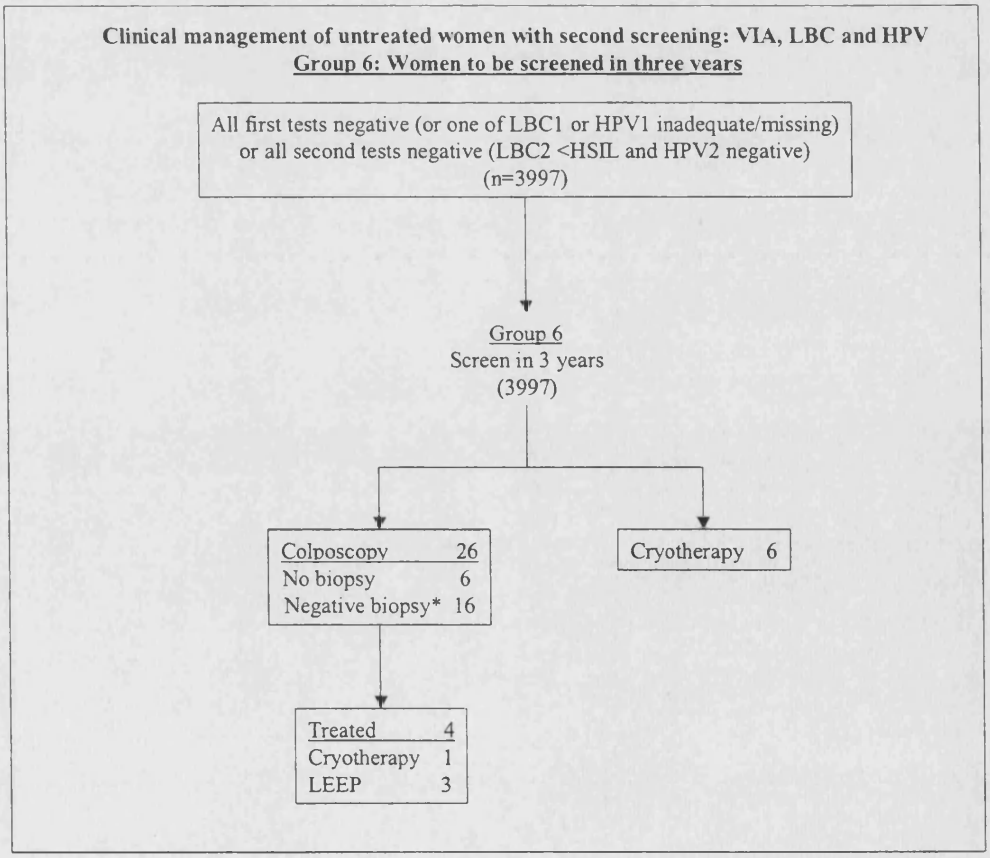
immediately treated with LEEP, and another was classified as suspected cancer and referred to INEN (no biopsy was collected during colposcopy).

The pathology results of the 22 women with biopsy were negative on 10 women (including one condyloma), LSIL on 4, HSIL on 3 and in five women the results are not available yet. None of these women have been treated so far.

Unfortunately, 156 women have not had colposcopy yet and therefore, are not fully evaluated.

Group 6: Women with first negative screening or untreated after initial screening who had second screening negative.

Figure 5.3.5. Clinical management of untreated women with second screening negative (LSIL or less on second LBC and second HPV negative).



* One biopsy was lost before reaching the laboratory.

Overall, a total of 3997 women were considered as negative, 3822 after initial screening and 175 after second screening.

Six women had cryotherapy, four of them after having a positive VIAM and a negative VIA (violation of protocol) and the other two after having a positive VIAM after an n unnecessary second positive VIA (violation of protocol).

Twenty-six women had colposcopy (violation of protocol), six of them were classified as negative on colposcopy and were not biopsied, 16 were biopsied but one biopsy sample was lost before reaching the pathology laboratory, the other 15 biopsies were negative. Four women who were colposcopically diagnosed with condyloma or mild dysplasia were treated, one was referred back to the general doctor for cryotherapy and the other three underwent LEEP.

5.3.1. Summary of treatment procedures

Overall 392 women have been treated, the majority with cryotherapy (84.2%) as it was offered as the first option to women.

Table 5.3.1.1. Summary of treatment procedures

Treatment	n	%
Cauterization	2	0.5
Cryotherapy	330	84.2
LEEP	41	10.5
Cold cone	7	1.8
Hysterectomy	4	1.0
Referred to INEN and treated*	8	2.0
Total treated	392	100.0

* Three other women were referred to INEN but rejected treatment.

Forty-one LEEPs were performed, 7 cold cones and four hysterectomies. Eleven women were referred to INEN with clinically confirmed or histologically confirmed cancer, but three of them rejected the treatment offered (radiotherapy). One of the women who had LEEP and another one who underwent cold conization had hysterectomies after treatment failure was confirmed.

5.4. Histology results

So far, 590 women have histology results, 57 of them had two biopsies and 8 had three different biopsies. Table 5.4.1 presents the histology results; when a woman has had more than one biopsy, the severest abnormality is reported.

Table 5.4.1. Histology results (as reported by INEN).

	Women with histology	
Histology result	n	%
Inadequate	16	2.7
Negative	53	9.0
Cervicitis	141	23.9
Metaplasia	71	12.0
Condyloma/HPV	155	26.2
Mild dysplasia	51	8.6
Moderate dysplasia	33	5.6
Severe dysplasia	17	2.9
Carcinoma in situ	31	5.2
Microinvasive cancer	4	0.7
Invasive carcinoma*	18	3.0
Invasive adenocarcinoma	1	0.2
Total	591	100.0

*One invasive cancer diagnosed clinically.

Sixteen women had inadequate samples, 13 of them were treated with cryotherapy, and the other three were biopsied during colposcopy. All this women have been considered "not fully evaluated". One woman had a biopsy, which was lost before reaching the laboratory, and 20 have been biopsied but their results have not been reported yet.

A total of 265 (45%) women had negative biopsies (normal, cervicitis, metaplasia), 155 (26%) had condyloma/HPV usually reported as koilocytotic changes, 51 (9%) had mild dysplasia and 104 (18%) had HSIL or worse, including 18 invasive carcinomas and one invasive adenocarcinoma. One of these invasive carcinomas was only clinically diagnosed and the patient was referred to the cancer hospital for treatment, but once in Lima, she refused to be examined. She has been considered as "with disease" for the following analysis.

Table 5.4.2. Age-specific rates of confirmed cervical lesions per 1000 screened women.

Age group	No. screened women	Rates of histologically confirmed cervical lesions				
		Mild Dysplasia n=51	Moderate Dysplasia n=33	Severe Dysplasia n=17	CIS n=31	Cancer n=23
<30	1542	11.7	9.1	3.2	4.5	1.3
30-34	1480	10.1	6.8	1.4	6.1	3.4
35-39	1251	9.6	2.4	4.0	4.8	6.4
40-44	776	6.4	2.6	2.6	7.7	5.2
>44	516	1.9	7.8	5.8	5.8	7.8
Total	5565	9.2	5.9	3.1	5.6	4.1

CIS: Carcinoma in situ

Table 5.4.2 presents the age-specific rates of confirmed cervical lesions per 1000 women. Those younger than 35 years had higher rates of mild

dysplasia and moderate dysplasia. Women over 35 years of age had higher rates of severe dysplasia, carcinoma in situ and cancer.

5.5. Sensitivity and Specificity

These results are based on 5539 women who had initial VIA and adequate first samples of LBC and HPV and are presented in three different sections, as estimations were performed.

5.5.1. Estimation of women with VIAM missing data

Step 1 (Figure 4.3.3.3.1):

Several multinomial logistic regression models were fitted to predict the missing VIAM outcomes using the initial cervical samples results. All these models are based on women who were positive on initial VIA and so, were referred to VIAM. Those who were negative on VIA but were examined with VIAM were excluded from this analysis (violation of protocol).

LBC7 (seven categories of initial LBC) and HPV1 as defined in 4.3.2 (e and k) were used as explanatory factors, being their reference groups: LBC negative and HPV negative. As for VIAM, the comparison group was VIAM negative.

First, a full model was fitted allowing for interactions, but none of them resulted significance, so a main effects model was fitted but HPV did not contribute to the prediction of VIAM result, and so a third model using only LBC7 was fitted.

The same steps were repeated considering LBC4 (four categories of LBC, as defined in 4.3.2g), once more the interaction was not significant and HPV did not contribute into the model. Table 5.5.1.1 presents a summary of the Log likelihood ratio test comparing the models.

Table 5.5.1.1. Log likelihood ratio tests to assess the fitting of models used to predict VIAM results.

	Fitted Model	Log likelihood	Par.	Comp.	LR test Chi-sq(df)	p-value
1(a)	LBC7*HPV1	-1172.5068	22[i]			
1(b)	LBC7 HPV1	-1178.4805	14	(a) vs (b)	11.95(8)	0.1536
1(c)	LBC7	-1180.6387	12	(b) vs (c)	4.32(2)	0.1155
1(d)	LBC4*HPV1	-1176.8161	14			
1(e)	LBC4 HPV1	-1181.2604	8	(d) vs (e)	8.89(6)	0.1799
1(f)	LBC4	-1183.5473	6	(e) vs (f)	4.57(2)	0.1016

Par=number of parameters. Comp=comparison.
(i). 26-4 parameters, two interactions were dropped due to collinearity.

The reference categories used in the models were VIAM negative, LBC7 or LBC4 negative, and HPV negative.

Interactions between LBC and HPV were not significant in either models 1(a) or 1(d), therefore only main effects models are presented.

Models based on LBC4 (Negative, LSIL, HSIL, Inadequate) fitted better than those with LBC7 (Negative, ASCUS/AGUS, LSIL, Moderate Dysplasia, Severe Dysplasia/CIS, Cancer, Inadequate), but model 1(c) is selected for further analysis because of being more informative (see Appendix, Table 5.6.1-5.6.2).

Table 5.5.1.2 presents the predicted probability of VIAM results by LBC expressed in percentages, using the Model 1 (c).

The doctor's interpretation of VIAM could not be affected by the LBC result because the latter was not available at the time of VIAM exam. In women who were VIA positive, the probability of having a negative result on VIAM decreased with increasing severity of the result on LBC. The probability of needing colposcopy after VIAM was higher for women with high-grade disease, and the more severe LBC, the more likely women would need colposcopy as a result of VIAM. This is confirmed by estimates from model 1(c) (see Appendix at the end of the chapter).

Table 5.5.1.2. Probability (%) of women with estimated VIAM result according to LBC (Model 1(c)).

LBC	VIAM Negative	VIAM Cryotherapy	VIAM Colposcopy	Total
Negative	59	27	14	100
ASCUS	50	24	26	100
LSIL	52	28	20	100
Moderate	39	32	29	100
Severe	29	24	47	100
Cancer	22	11	67	100
Inadequate	68	17	15	100

After choosing model 1(c), predictive values were obtained for the expanded data and used to weight the records generated after the expansion, assigning the estimated probability of having each result to the appropriate pseudo-observation for each woman with original VIAM missing data. Variables related to VIAM were then updated (VIA_M1, VIA_MM1 and clinical management group).

5.5.2. Estimation of women with second screening tests results missing

Step 2 (Figure 4.3.3.3.1):

After expanding three times the data with missing values on second screening tests results (SECSCR=Missing, 4.3.2 (v)), several multinomial logistic regression models were fitted to predict the missing outcomes using the initial cervical samples results. All these models were based on women who required second screening (negative on initial VIA_M, ASCUS, AGUS, condyloma/HPV or mild dysplasia on LBC or negative on LBC but positive on HPV). Those who had other previous initial results but still had second screening were excluded from this analysis (unnecessary second screening). Only women who had all three second screening results (VIA_M, LBC and HPV) were used to fit the models.

LBCRES and HPV1 as defined in 4.3.2 (h and k) were considered explanatory factors, being the reference groups: LBC negative and HPV negative. As for SECSCR, negative was the comparison group.

First, a full model was fitted allowing for interactions, but the model was unstable and none of the interactions resulted significance. Then, a main effects model was fitted. Both LBCRES and HPV1 appeared to have an independent effect on the probability of having a colposcopy as a result of second screening but not of them affected the probability of having a cryotherapy after second screening (as compared with being negative on second screening).

A third model excluding HPV1 was fitted and as expected, the Likelihood Ratio test was significant, implying that HPV1 contributed to the prediction of second screening results and should not be taken out from the model.

Table 5.5.2.1 presents a summary the Log likelihood ratio test comparing the models. The interaction model is not included in this table, as it did not contribute to the analysis.

Table 5.5.2.1. Log likelihood ratio tests to assess the fitting of models used to predict second screening results.

	Fitted Model	Log likelihood	LR test Comparison	Chi-	p-value
2(a)	LBCRES HPV1	-219.5003			
2(b)	LBCRES	-227.7284	(a) vs (b)	16.45(2)	0.003

In general, all models have problems with estimation due to the small sample used, only 287 observations were considered for estimating three different outcomes. Because women requiring second screening were selected using two main combinations of LBCRES and HPV1 results, the interactions are not modelled properly. Despite these problems, both LBCRES and HPV1 contributed to estimate the probability of having a

certain second screening result, and model 2(a) was chosen for predicting second screening missing results (see Appendix: Table 5.6.3).

Table 5.2.2.2 shows the predicted probability (expressed in %) of second-stage results by LBC and HPV testing, using Model 2(a).

Being HPV negative on first samples increase the probability of discharge after second screening, the probability of treatment with cryotherapy was minimum (very few women with results in this category); while being HPV positive or having had LSIL on LBC increases the probability of referral to colposcopy.

Table 5.2.2.2. Probability (in %) of second-stage results by LBC and HPV first results (Model 2(a)).

	Discharge		Treat		Refer	
LBC	HPV neg	HPV pos	HPV neg	HPV pos	HPV neg	HPV pos
Negative	78	48	2	2	20	50
ASCUS	81	51	*	*	19	49
LSIL	62	29	6	7	33	64
Inadequate	80	50	*	*	20	50

* Probabilities very small (almost zero).

After choosing model 2(a), predictive values were obtained and used to weight the records generated after the expansion (each one with a different second screening result), assigning a different probability of having a particular result to each woman with original second screening missing data. Variables related to second screening were then updated (SECSCR and clinical management group).

5.5.3. Estimation of disease status for women "not fully evaluated"

Step 3 (Figure 4.3.3.1):

Several survey logistic models (weighted logistic regression) were used for analysis.

Once more, data on observations with missing disease status (HSIL=Missing, 4.3.2 (w)) were expanded to create new "observations" for each old observation.

First, empirical predictive values (***p1***) of disease status were obtained by counting the original number of women with HSIL in each of 48 categories generated by three variables: VIA_MM1, LBC6 and HPV1. New weights were then calculated using these empirical estimates (***w1***); these results are to be present at the end of this section.

Then, weighted logistic regression models were fitted to predict the missing outcomes using the disease status of women fully evaluated. Two sets of models were used; the first one using two separate models for women who did not required second screening and those who needed second screening; and the second one using one model for all women in the study.

For the models on women who did not need second screening or the overall model (all women in the study); VIA_MM1 (four categories of VIA and VIAM combined), LBC6, HPV1, CCH_O_LB (HSIL on conventional cytology and not on LBC), and separate interactions for VIA1 and LBCHSIL, VIA1 and HPV1 and LBCHSIL and HPV1, were used as explanatory variables (4.3.2 (d), (f), (k), (m), (n), (o), (p)). The reference comparison outcome was "no HSIL", and the reference levels for the other variables were VIA negative for VIA_MM1, LBC negative for LBC6, and HPV negative for HPV1. Interactions were positive if both tests were positive and negative otherwise, and this negative category was used as the reference group when modelling. For the models on women who needed second screening, LBC2 and HPV2 (4.3.2 (t), (u)) were used as

explanatory variables with LBC2 negative and HPV2 negative being the reference groups.

Of all models fitted, six are detailed in the Appendix of these chapter (Tables 5.6.4-5.6.9), two sets of models for women requiring and not requiring second screening, and two models for all women in the study.

Models 3(a1) and 3(b1) differ only in the inclusion or not of a factor evaluating the effect of conventional cytology HSIL but less than HSIL on LBC (CC_O_LB) in predicting histologically confirmed HSIL. The overall results are quite similar: Women who underwent cryotherapy or colposcopy as a result of initial VIAM; those who ASCUS on LBC, HSIL on CC (only Model 3(a1)) or LBC, or Cancer on LBC; and those who were positive on first HPV testing had a higher risk of having high-grade disease.

In Models 3(a2) and 3(b2) both second HPV and second LBC are used to estimate the probability of having HSIL in women needing second screening. Having a second positive result on HPV sample positive did not predict HSIL (p-value=0.239), while having a second positive LBC sample increase the probability of having high-grade disease (p-value=0.049).

Several alternative models were fitted in the subpopulation of women who needed second screening, VIA2, VIAM2, VIA_M2, and combinations of these with LBC2 and HPV2, but the small number of results available made models very unstable, and no good estimation of the effects of such factors were obtained (data not presented).

For the purpose of estimating measure of performance Model 3(a1) was used, because of CCH_O_LB (HSIL on CC and not on LBC) being informative. We decided to use Model 3(a2) a priory, since the HPV result could only be positive or negative, while when considering a woman negative on a second LBC, she could have low-grade disease.

Again, Models 3(c) and 3(d) only differed in the inclusion or not of CC_O_LB. Women who underwent colposcopy as a result of initial

VIAM; those who had HSIL on CC (Model 3(c)) or LBC, or Cancer on LBC; and those who were positive on first HPV testing had a higher risk of having high-grade disease.

Once again, Model 3(c) was used for estimation of measures of tests performance because CC_O_LB was informative enough to be included.

After selecting Models 3(a1), 3(a2) and 3(c), the predictive values for each model were obtained. For Models 3(a1) and 3(a2), the predictive values were combined (***p2***) and were used to generate another set of weights (***w2***). The predictive values (***p3***) from Model 3(c) were also used to generate the last set weights (***w3***).

These two sets of weights (***w2*** and ***w3***), together with those from the empirical estimation (***w1***), were combined with the previous weights obtained when estimating screening missing data (VIAM and second screening), and were used to obtain three different sets of estimates of measures of tests performance.

Table 5.5.3.1 shows a summary of the probability of having disease given the results of screening tests using the three set of predictive values ***p1***, ***p2*** and ***p3*** (Step 4: Figure 4.3.3.3.1).

The first two rows of the table are the observed number of women "fully evaluated" and the observed proportion of women with confirmed high-grade disease in each category according to certain combination of screening tests results. The third row is the estimated number of women in each category after allocating women with original missing screening tests results. The last three rows correspond to the probability of having high-grade disease (shown as a percentage) obtained from empirical estimation (***p1***), after fitting models 3(a1) and 3(a2) (***p2***), and model 3(c) (***p3***).

Table 5.5.3.1: Probability of detecting disease given screening test results if all women complied with follow-up evaluation.

		HPV Negative						HPV Positive					
LBC result:		Neg	ASCUS	LSIL	HSIL	Cancer	Inad	Neg	ASCUS	LSIL	HSIL	Cancer	Inad
VIA	n	3074	48	149	11		196	61	11	31	40	5	6
Negative	p	0	2.1	1.3	18.2		0	3.3	18.2	6.5	45	100	16.7
	n	3074	91	341	21	1	196	229	27	112	69	6	27
	p1	0*	2.1	1.3	18.2	93.3	0	3.3	18.2	6.5	45	100	16.7
	p2	0.1	5.7	6.9	10.4	72	0	10.2	15.3	14.4	47.9	95.3	12.1
	p3	0.5	6.1	0.4	19.2	84.2	0.2	11.0	63.4	10.1	46.3	95.1	6.6
VIA	n	496	5	13	2		41	9	1	2	11	2	2
Positive	p	0	0	0	0		0	11.1	0	0	27.3	100	0
VIAM	***n***	541	19	58	5		43	51	2	24	18	2	10
Negative	***p1***	0	0	0	0		0	11.1	0	0	27.3	100	0
	p2	0	5.0	7.3	4.6		0	10.8	7.4	13.5	27.6	89.4	9.2
	p3	0.2	2.4	0.1	8.2		0.1	4.5	36.9	3.1	24.6	87.9	1.9

n=Number of women "fully evaluated" in that group. Note that women testing negative on all screening tests were deemed to be fully evaluated even though they either did not have colposcopy or a biopsy.
p=percentage of women with HSIL in that group.
n=Estimated number of women in that group, after allocation of missing screening results.
p1, p2, p3: Estimated percentage of women with HSIL with: ***p1***=empirical, ***p2***=combining models 3(a1) and 3(a2), ***p3***=model 3(c); there were 57, 113, and 96 imputed cases of HSIL (or worse) lesions under p1, p2 and p3 estimates, respectively.
* There were no cases of CIN III diagnosed in 3074 women negative on all three tests, but only 4 had colposcopy.
.....Continued on Page 151

.....continuing from Page 150

Table 5.5.3.1: Probability of detecting disease given screening test results if all women complied with follow-up evaluation.

		HPV Negative						HPV Positive					
LBC result:		Neg	ASCUS	LSIL	HSIL	Cancer	Inad	Neg	ASCUS	LSIL	HSIL	Cancer	Inad
VIAM	n	190	9	23	4		12	30		14	14	1	1
Cryotherapy	p	3.2	0	0	50		0	13.3		28.6	35.7	100	0
	n	232	10	27	4		13	33		17	15	1	1
	p1	3.2	0	0	50		0	13.3		28.6	35.7	100	0
	p2	2.4	7.6	2.3	49		1.6	17.8		24.1	29	90.1	12.7
	p3	2.3	22.6	1.6	26.9		1.0	17.8		20.0	33.9	92.0	8.3
VIAM	n	113	6	13	3		5	10	2	14	15	6	5
Colposcopy	p	7.1	16.7	15.4	33.3		0	50	100	35.7	60	83.3	40
	n	127	9	16	4		6	11	2	16	18	7	5
	p1	7.1	16.7	15.4	33.3		0	50	100	35.7	60	83.3	40
	p2	6.7	19.3	6.4	73.6		4.6	43.5	67.6	44.1	54.2	96.3	40.8
	p3	6.3	45.7	4.4	51.4		2.7	43.4	88.7	37.3	59.6	97.1	32.8

n=Number of women "fully evaluated" in that group. Note that women testing negative on all screening tests were deemed to be fully evaluated even though they either did not have colposcopy or a biopsy.
p=percentage of women with HSIL in that group.
n=Estimated number of women in that group, after allocation of missing screening results.
p1, p2, p3: Estimated percentage of women with HSIL with: ***p1***=empirical, ***p2***=combining models 3(a1) and 3(a2), ***p3***=model 3(c); there were 57, 113, and 96 imputed cases of HSIL (or worse) lesions under p1, p2 and p3 estimates, respectively.

Comparing the observed number of women in each cell (generated by different set of screening tests results) with the estimated one after allocating women with incomplete screening; for instance, among women who were LBC and HPV negative: 44% of women with missing VIAM were assigned a negative result, 42% a positive requiring cryotherapy result and 14% a positive requiring colposcopy result.

Women with missing VIAM or missing second screening were in general more allocated into categories with LBC ASCUS/AGUS and LSIL results, independently of HPV results.

As expected, among women with HPV positive and VIAM positive results, the higher the diagnosis on LBC the less number of women allocated.

With regard to the probability of having disease obtained by different methods, once again, in those with HPV and VIAM positive results the higher the diagnosis on LBC the less difference between the three estimates of the probability of disease, especially among those with carcinoma on LBC for whom almost all probabilities were over 85%.

Overall, the empirical probabilities tended to be smaller than the modelling ones among women with HPV negative and LBC less than HSIL results, and larger or similar among those being HPV positive and having HSIL or worse lesions.

Comparing the estimates from modelling, model 3(c) estimates were larger than the empirical ones and those of models 3(a1) and 3(a2) combined among VIA_M negative women with ASCUS who were HPV positive, but, model 3(c) estimates were smaller than the other modelling estimates among women HPV negative, with LSIL and a VIAM cryotherapy results.

Because of these differences between estimates of disease status, the three estimates were used to calculate measures of tests performance.

Table 5.5.3.2 presents the sensitivity, specificity, and PPV of each screening test and their confidence intervals for each of the three estimates of disease status previously obtained.

Table 5.5.3.2: Measures of performance of screening tests.

Test		Sens	(95%CI)	Spec	(95%CI)	PPV	(95%CI)
VIA	***p1***	52.5	(41.1,64.0)	76.6	(76.1,78.4)	6.3	(4.9,8.2)
	p2	44.0	(34.4,58.6)	76.5	(74.2,76.7)	7.1	(5.1,8.4)
	p3	41.4	(30.6,59.5)	76.4	(74.2,76.6)	6.5	(4.7,7.8)
Combined	***p1***	41.1	(32.0,52.2)	91.4	(90.6,92.1)	12.5	(9.8,15.5)
VIA&VIAM	***p2***	30.5	(20.2,49.9)	91.3	(90.6,92.2)	12.5	(9.8,15.5)
	p3	31.7	(21.7,50.1)	91.3	(90.6,92.2)	12.6	(9.8,15.5)
LBC ≥ASCUS	***p1***	68.1	(57.5,79.0)	84.6	(83.6,85.7)	11.7	(9.4,14.7)
	p2	68.9	(61.3,78.1)	85.2	(84.0,86.4)	15.9	(10.5,21.9
	p3	64.3	(44.8,82.6)	84.9	(83.7,86.0)	14.4	(9.7,18.7)
LBC ≥HSIL	***p1***	47.0	(37.9,58.3)	98.2	(97.8,98.6)	44.3	(35.2,54.5
	p2	34.9	(22.5,55.8)	98.2	(97.8,98.6)	44.4	(34.1,53.3
	p3	36.3	(25.4,55.9)	98.2	(97.8,98.6)	44.6	(34.4,53.7
HPV	***p1***	77.7	(69.3,84.9)	89.3	(88.4,90.5)	17.9	(13.8,22.6
	p2	72.2	(65.2,78.8)	89.8	(88.5,91.1)	22.4	(14.1,32.2
	p3	79.9	(61.7,89.1)	90.0	(88.4,91.5)	23.9	(13.6,34.7

p1: empirical estimates.
p2: estimates combining models 3(a1) and 3(a2).
p3: estimates using model 3(c).
Measures of tests performance were based on 104 observed cases of high-grade disease (HISL or worse), and in 57, 113, and 96 estimated cases from p1, p2 and p3, respectively.

Sensitivity depends on prevalence of disease, therefore the estimates of sensitivity varied according to the predictive value used (***p1***, ***p2***, ***p3***). This was not the case for specificity and PVV estimates. In general, the empirical estimates (using ***p1***) of sensitivity were larger than the other two, except for HPV, where the ***p3*** estimate was the largest. Overall, estimates

from modelling (***p2*** and ***p3***) were quite similar, but because the bootstrapping confidence intervals obtained after combining Models 3(a1) and 3(a2) (***p2***) were smaller than the others; for purposes of reporting results, ***p2*** estimates would be used.

The sensitivity of VIA was 44% (CI: 34,59), its specificity was 77% (CI: 74,77) and its PPV was 7% (CI: 5,8). The sensitivity of combined VIA & VIAM was 31% (CI: 20,50), its specificity was 91% (CI: 91,92) and its PPV was 13% (CI: 10,16). Incorporating VIAM after VIA, increased the specificity of the visual technique, but reduced its sensitivity.

The sensitivity of LBC using a low threshold (ASCUS or worse) was 69% (CI: 61,78), its specificity was 85% (CI: 84,86) and its PPV was 16% (CI: 11,22); and when using a high-threshold (moderate dysplasia or worse lesions) the sensitivity of LBC was 35% (CI: 23,56), its specificity was 98% (CI: 98,99) and its PPV was 44% (CI: 34,53). A higher-threshold for LBC ensured a gain in specificity and increased the PPV, but with a substantial loss of sensitivity.

Finally, the sensitivity of HPV testing (HC-II) was 72% (CI: 65,79), its specificity was 90% (CI: 89,91) and its PPV was 22% (CI: 14,32).

Overall, HPV had better sensitivity than the other tests, but it had low specificity. Both VIA, and combined VIA & VIAM had very low sensitivities, VIA had also very low specificity but when combined with VIAM the specificity increased by 15%.

Table 5.5.3.3 presents the sensitivity, specificity and PPV of VIA combined with LBC or HPV testing.

The idea of combining VIA with another screening test was based on the assumption that VIA would have high sensitivity but poor specificity. In fact, the specificity of VIA combined with either LBC≥ASCUS or LBC≥HSIL or HPV testing, was over 95%, at least 19% improvement. However, in this study, VIA had very poor sensitivity; nevertheless, the combination of VIA with HPV yielded a sensitivity of 31% (CI: 23,41); which was higher than that of VIA combined with LBC.

Table 5.5.3.3: Measures of performance of combinations of screening tests.

Combination of tests		Sens	(95%CI)	Spec	(95%CI)	PPV	(95%CI)
Combined	*p1*	31.2	(23.0,40.9)	95.9	(95.5,96.8)	18.5	(14.1,25.5)
VIA &	*p2*	27.4	(20.3,38.3)	96.1	(95.2,96.5)	21.9	(15.1,26.6)
LBC≥ASCUS	*p3*	25.7	(17.0,39.2)	95.9	(95.1,96.4)	19.9	(13.6,25.1)
Combined	*p1*	21.0	(14.4,29.6)	99.3	(99.0,99.5)	45.8	(33.0,59.7)
VIA &	*p2*	15.8	(9.3,25.6)	99.3	(99.0,99.5)	46.4	(32.4,57.4)
LBC≥HSIL	*p3*	16.3	(10.2,26.4)	99.3	(99.0,99.5)	46.3	(32.6,57.8)
Combined	*p1*	37.9	(28.0,48.8)	96.8	(96.4,97.7)	26.3	(20.0,37.2)
VIA & HPV	*p2*	30.5	(22.8,41.4)	96.9	(96.2,97.3)	28.6	(20.3,34.9)
	p3	29.2	(20.4,42.8)	96.8	(96.1,97.3)	26.4	(18.9,33.8)

p1: empirical estimates.
p2: estimates combining models 3(a1) and 3(a2).
p3: estimates using model 3(c).

5.6. APPENDIX: Results

Table 5.6.1 MODEL 1(b): Main effects: LBC7 and HPV1

Multinomial logistic regression — Number of obs = 1243
LR chi-sq (14) = 45.56
Prob>chi-sq < 0.001
Log likelihood=-1178.4805 — Pseudo R-sq = 0.0190

	Coefficient	St.	Z	P>|z|	[95% CI]	
VIAM=Cryotherapy						
LBC_ASC	0.0535	0.4122	0.13	0.897	-0.7546	0.8615
LBC_LSIL	0.0974	0.2154	0.45	0.651	-0.3247	0.5196
LBC_Mod	0.4075	0.3879	1.05	0.293	-0.3527	1.1678
LBC_Sev	0.2758	0.6978	0.40	0.693	-1.0918	1.6434
LBC_Ca	-0.1942	1.2397	-0.16	0.876	-2.6240	2.2356
LBC_Inad	-0.5824	0.3220	-1.81	0.070	-1.2136	0.0487
HPV_Pos	0.3365	0.1964	1.71	0.087	-0.0721	0.7214
Constant	-0.8354	0.0798	-10.47	<0.001	-0.9919	-0.6790
VIAM=Colposcopy						
LBC_ASC	0.8195	0.4035	2.04	0.042	0.0288	1.6103
LBC_LSIL	0.4334	0.2457	1.76	0.078	-0.0482	0.9505
LBC_Mod	0.9673	0.4062	2.38	0.017	0.1711	1.7634
LBC_Sev	1.5911	0.6134	2.59	0.009	0.3889	2.7933
LBC_Ca	2.2197	0.8473	2.62	0.009	0.5591	3.8804
LBC_Inad	-0.0941	0.3490	-0.27	0.787	-0.7781	0.5900
HPV_Pos	0.3804	0.2292	1.66	0.097	-0.0687	0.8296
Constant	-1.5015	0.1022	-14.69	<0.001	-1.7019	-1.3012

Table 5.6.2. MODEL 1(c): Main effects: LBC7

Multinomial logistic regression — Number of obs = 1243
LR chi-sq (12) = 41.25
Prob>chi-sq < 0.001
Log likelihood=-1180.6387 — Pseudo R-sq = 0.0172

	Coefficient	St.	Z	P>|z|	[95% CI]	
VIAM=Cryotherapy						
LBC_ASC	0.0546	0.4119	0.13	0.887	-0.7528	0.8619
LBC_LSIL	0.1846	0.2086	0.89	0.366	-0.2241	0.5933
LBC_Mod	0.6076	0.3690	1.65	0.097	-0.1157	1.3309
LBC_Sev	0.5786	0.6752	0.86	0.388	-0.7448	1.9021
LBC_Ca	0.1086	1.2272	0.09	0.927	-2.2966	2.5138
LBC_Inad	-0.5453	0.3207	-1.70	0.092	-1.1739	0.0833
Constant	-0.8018	0.0770	-10.41	<0.001	-0.9528	-0.6508
VIAM=Colposcopy						
LBC_ASC	0.8208	0.4030	2.04	0.042	0.0310	1.6107
LBC_LSIL	0.5331	0.2372	2.25	0.025	0.0682	0.9980
LBC_Mod	1.1944	0.3815	3.13	0.002	0.4467	1.9421
LBC_Sev	1.9327	0.5786	3.34	0.001	0.7986	3.0667
LBC_Ca	2.5613	0.8225	3.11	0.002	0.9493	4.1733
LBC_Inad	-0.0515	0.3474	-0.15	0.882	0.7323	0.6294
Constant	-1.4627	0.9887	-14.79	<0.001	1.6564	-1.2689

Table 5.6.3. MODEL 2(a): Main effects: LBCRES and HPV1

Multinomial logistic regression — Number of obs = 287
LR chi-sq (8) = 32.23
Prob>chi-sq = 0.0001
Log likelihood=-219.5022 — Pseudo R-sq = 0.0684

	Coefficient	St. error	Z	P>|z|	[95% CI]	
Second screening=Cryotherapy						
LBC_ASC	-30.3314	4346595	-0.00	1.000	-8519200	8519139
LBC_LSIL	1.5592	0.9633	1.62	0.106	-0.3287	3.4472
LBC_Inad	-30.5802	1.4798	-0.00	1.000	-1.94e+07	1.94e+07
HPV_Pos	0.9070	0.7633	1.19	0.235	-0.5899	2.4029
Constant	-3.9274	1.0521	-3.73	<0.001	-5.9895	-1.8653
Second screening=Colposcopy						
LBC_ASC	-0.0809	0.4975	-0.16	0.871	-1.0561	0.8942
LBC_LSIL	0.7271	0.3812	1.91	0.056	-0.0199	1.4742
LBC_Inad	-0.0476	0.7400	-0.06	0.949	-1.4981	1.4028
HPV_Pos	1.4103	0.3578	3.94	<0.001	0.7089	2.112
Constant	-1.3627	0.4192	-3.35	0.001	-2.1842	-0.5411

Table 5.6.4. MODEL 3(a1): Main effects with special interactions for women who did not require second screening

Survey logistic regression — Number of obs = 4704
Population size = 5539.43
F(13, 4691) = 18.27
Subpopulation No. of obs=4372 — Prob>F < 0.001
Subpopulation Size=4813.41

	Coefficient	St. error	t	P>|t|	[95% CI]	
HGSIL						
VIAM_neg*	-0.8835	0.6522	-1.35	0.176	-2.1621	0.3951
VIAM_Cryo	3.6489	0.6034	6.05	<0.001	2.4661	4.8318
VIAM_Colp	4.7121	0.5988	7.87	<0.001	3.5383	5.8860
CC_HSIL	2.7837	1.3122	2.12	0.034	0.2112	5.3563
LBC_ASC	1.2027	0.5447	2.21	0.027	0.1349	2.2705
LBC_LSIL	-0.0583	0.5095	-0.11	0.909	-1.0571	0.9405
LBC_HSIL	5.1877	1.0986	4.72	<0.001	3.0339	7.3415
LBC_Ca	8.2867	1.5547	5.33	<0.001	5.2388	11.3346
LBC_Inad	-0.4012	0.6380	-0.63	0.529	-1.6520	0.8496
HPV1	5.0933	1.2691	4.01	<0.001	2.6053	7.5813
VIA_LBHG	-1.5343	1.2392	-1.24	0.216	-3.9637	0.8951
VIA_HPV1	-2.9270	1.2270	-2.39	0.017	-5.3324	-0.5215
LBHG_HPV	-3.0205	1.0060	-3.00	0.003	-4.9928	-1.0481
Constant	-7.3428	0.5016	-14.64	<0.001	-8.3261	-6.3595

* VIAM_neg includes women VIA positive and VIAM negative.

Table 5.6.5 MODEL 3(a2): HPV2 on women who required second screening

Survey logistic regression — Number of obs = 323
Population size = 717.02
F(1, 322) = 1.39
Subpopulation No. of obs=323 — Prob>F = 0.2385
Subpopulation Size=717.02

	Coefficient	St. error	t	P>|t|	[95% CI]	
HGSIL						
HPV2	1.1691	0.9900	1.18	0.239	-0.7786	3.1169
Constant	-2.7684	0.7838	-3.53	<0.001	-4.3104	-1.2264

Table 5.6.6. MODEL 3(b1): Main effects with special interactions for women who did not require second screening (excluding CC HSIL)

Survey logistic regression — Number of obs = 4704
Population size = 5539.43
F(12,4692) = 25.66
Subpopulation No. of obs=4372 — Prob>F < 0.001
Subpopulation Size=4813.41

	Coefficient	St. error	t	P>|t|	[95% CI]	
HGSIL						
VIAM_neg	-0.9328	0.6563	-1.42	0.155	-2.2193	0.3538
VIAM_Cryo	3.1733	0.5628	5.64	<0.001	2.0699	4.2767
VIAM_Colp	4.2826	0.5553	7.71	<0.001	3.1939	5.3712
LBC_ASC	1.3339	0.5523	2.42	0.016	0.2511	2.4167
LBC_LSIL	0.2422	0.4558	0.53	0.595	-0.6514	1.1358
LBC_HSIL	4.1933	1.4067	2.98	0.003	1.4355	6.9510
LBC_Ca	7.3128	1.7852	4.10	<0.001	3.8130	10.8125
LBC_Inad	-0.0976	0.6218	-0.16	0.875	-1.3166	1.1215
HPV1	6.7680	1.0671	6.34	<0.001	4.6759	8.8601
VIA_LBHG	0.1514	1.0480	0.15	0.885	-1.9032	2.2061
VIA_HPV1	-4.3414	1.1301	-3.84	<0.001	-6.5570	-2.1259
LBHG_HPV	-3.9833	1.1795	-3.38	0.001	-6.2957	-1.6710
Constant	-7.0066	0.4138	-16.94	<0.001	-7.8179	-6.1953

Table 5.6.7. MODEL 3(b2): LBC2 for women who required second screening

Survey logistic regression — Number of obs = 315
Population size = 706.67
F(1, 314) = 3.90
Subpopulation No. of obs=315 — Prob>F = 0.0492
Subpopulation Size=706.67

	Coefficient	St. error	t	P>|t|	[95% CI]	
HGSIL						
LBC2	1.9364	0.9809	1.97	0.049	0.0064	3.8665
Constant	-2.9371	0.7121	-4.12	<0.001	-4.3382	-1.5360

Table 5.6.8. MODEL 3(c): Main effects with special interactions for all women in the study

Survey logistic regression

Number of obs = 4704
Population size = 5539.43
F(13, 4691 = 13.66
Prob>F < 0.001

	Coefficient	St. error	t	P>|t|	[95% CI]	
HGSIL						
VIAM_neg	-0.9733	0.6023	-1.62	0.106	-2.1541	0.2075
VIAM_Cryo	1.5143	0.7658	1.958	0.048	0.0130	3.0156
VIAM_Colp	2.57	0.7624	3.37	0.001	1.0766	4.0660
CC_HSIL	2.9189	0.8707	3.35	0.001	1.2119	4.6259
LBC_ASC	2.5311	1.2984	1.95	0.051	-0.0144	5.0767
LBC_LSIL	-3.8692	0.7270	-0.53	0.595	-1.8122	1.0384
LBC_HSIL	3.8363	0.7192	5.33	<0.001	2.4263	5.2462
LBC_Ca	6.9458	1.3158	5.28	<0.001	4.3663	9.5253
LBC_Inad	-0.8681	0.6659	-1.30	0.192	-2.1736	0.4374
HPV1	3.1807	1.0876	2.92	0.003	1.0484	5.3130
VIA_LBHG	-1.0802	0.7032	-1.54	0.125	-2.4589	0.2985
VIA_HPV1	-0.9524	1.0533	-0.90	0.366	-3.0173	1.1126
LBHG_HPV	-1.8928	0.9781	-1.94	0.053	-3.8104	0.2480
Constant	-5.2735	0.5574	-9.46	<0.001	-6.3661	-4.1808

Table 5.6.9. MODEL 3(d): Main effects with special interactions for all women in the study (excluding CC HSIL)

Survey logistic regression

Number of obs = 4704
Population size = 5539.43
F(12,4692) = 14.33
Prob>F < 0.001

	Coefficient	St. error	t	P>|t|	[95% CI]	
HGSIL						
VIAM_neg	-1.0174	0.5986	-1.70	0.089	-2.1909	0.1561
VIAM_Cryo	1.5024	0.7665	1.96	0.050	-0.0003	3.0052
VIAM_Colp	2.5742	0.7593	3.39	0.001	1.0857	4.0628
LBC_ASC	2.5560	1.2863	1.99	0.047	0.0342	5.0778
LBC_LSIL	-0.0809	0.6644	-0.12	0.906	-1.4227	1,2608
LBC_HSIL	3.8924	0.7154	5.44	<0.001	2.4898	5.2949
LBC_Ca	7.0115	1.3182	5.32	<0.001	4.4273	9.5957
LBC_Inad	-0.3823	0.7226	-0.53	0.597	-1.7983	1.0336
HPV1	3.2428	1.0608	3.06	0.002	1.1632	5.3224
VIA_LBHG	-1.1494	0.6865	-1.67	0.094	-2.4953	0.1964
VIA_HPV1	-0.8733	1.0459	-0.83	0.404	-2.9238	1.1772
LBHG_HPV	-1.9795	0.9588	-2.06	0.039	-3.8591	-0.0999
Constant	-5.3011	0.5619	-9.43	<0.001	-6.4028	-4.1995

6. DISCUSSION

6.1. Introduction

Ideally, in a screening study all participants undergo colposcopy (with biopsy if indicated), so that they would be fully evaluated (those with disease would be histologically confirmed); in this way, no verification bias is introduced to the estimation of sensitivity and specificity of the screening tests.

The hypothesis of our study was that a two-stage process in which women had VIA and those positive on VIA had a second test, either LBC or HPV testing was a cost-effective approach to cervical screening in San Martin, Peru. The idea was that VIA would be highly sensitive, but that it would lack specificity. The hypothesised 20% of women positive on VIA would be told that their initial screening was positive and that should the sample sent to the laboratory also test positive, it would be very important for them to return to the clinic for treatment. It was hoped that presenting the results in this way would reduce the dropout rate. In the recently published HART study, the percentages of women declining colposcopy were 38% if they were negative on both HPV and cytology, 29% if they were positive on HPV and/or had borderline cytology, 8% if they had mild dyskaryosis, 2% if they had moderate dyskaryosis and 0% if they had severe dyskaryosis or worse lesions on cytology. These data confirmed our prior belief that the rate of dropout before colposcopy is strongly related to the screening results presented to women. Those who are told that it is very likely that they have disease that needs treating will generally attend for colposcopy, those who are told that it is important to rule out the possibility of disease are more likely to dropout.

We screened 5565 women with VIA, LBC, HC-II and conventional cytology, but only 580 women had histological confirmation of their disease status. Evidence from other studies suggests that the prevalence of HSIL in those testing negative on all four tests will be so low as to make taking random biopsies unnecessary and possibly unethical. For instance,

in a study in China, Belinson and colleagues [172] did not detect any CIN II or worse lesions among 1332 women who had biopsies taken and were later found to have tested negative on both ThinPrep Pap and HPV testing.

The design of this study, then, included the use of VIA, LBC and HC-II. However, due to the Ministry of Health regulations, conventional cytology was also carried out, so our cohort of 5565 women had in theory at least four screening tests (those positive on VIA had VIAM as well).

Our study population was composed of all women between 25 and 49 years of age accepting cervical screening and willing to have additional cervical samples collected, in the region of San Martin. In the year 2000, there were 102276 women within the age-range 25-49 overspread in an area of 51253 square kilometres (numbers based on projections of the national population census of 1993). These figures show how difficult it is to establish a screening programme in the region. Furthermore, women will not accept screening or treatment without consent of their male partners, who in the first place, needed to be convinced.

The region of San Martin includes a university where one of the most popular careers is midwifery. This has contributed to the establishment of a good health network, where a large number of midwives lead health community teams, they are the ones who locate patients and convince them to undergo treatment. These midwives were a key part in this study, without them the number of women lost-to-follow-up would have been striking. It is worth to mention that the Program for Appropriate Technology in Health, PATH, established a network using these teams and community leaders who organised information meetings and helped to trace women needing rescreening or treatment.

VIA is a simple test and is easy to learn. Its major advantage is that results are available immediately. It was expected that women who tested positive on VIA, would undergo VIAM immediately. But this only happened in 9% of the cases, and VIAM was performed even after one year in 5% of the cases. The results from conventional cytology were available within 6 months of collection (this has actually been improved since 2002).

Additional samples for LBC and HC-II were processed outside the region; this generated long delays on clinical management decisions, especially in determining who needed second screening. LBC results were available within one or two months after collection and HPV testing only after 3 to 4 months.

Another main operational problem of the study was the lack of colposcopists; indeed, it was one of the main reasons for not attempting to increase the number of women evaluated with colposcopy. At the beginning of the study there were only two trained colposcopists in the region, one in Moyobamba covering the north, and one in Tarapoto covering the centre and the south. But despite efforts of organising colposcopy clinics, long delays on being colposcopically or histologically diagnosed were unavoidable.

As, stated before, the fact that only positive women were colposcopically evaluated or treated, introduced verification biased to the study. One approach to overcome the lack of complete evaluation was to rescreen women who were positive only on Hybrid-Capture II or who were positive on LBC but had low-grade disease (including ASCUS, AGUS and condyloma/HPV). This second screening contributed to the completion of disease status of at least 400 women, but major efforts were required to recall women, rescreen them and evaluate them completely, and of course, there were a number of lost-to-follow-up. Nevertheless, the complete evaluation of these extra 400 women improved the evaluation of both techniques, LBC and HC-II, but complicated the design and the statistical analysis.

6.2. Participants

Not every woman recruited in the TATI project was invited to participate in this study. Between February and December 2001, only 5596 of 13426 women recruited by the TATI project, were also screened with LBC or HPV testing. Women were invited to participate in this study if resources were available at the time of visit, and only those who signed the informed

consent for additional samples were recruited. A number of problems affected the recruitment during the study; mainly, changes in directives in the Health Direction of San Martin (DIRES) that meant changes in health policy and health priorities in the region. Midwives did their best to screen with VIA as many residents as possible, but were restricted by the current health policy. From time to time, new authorities would consider the TATI project, and hence this study, as the least important intervention in the region, and clinical midwives were told to use most of their time in activities other than cervical screening. Also, malaria outbreaks in the region meant concentration of resources to control it. In general, midwives tried to perform VIA once per week, screening an average of 20 women. But explaining the benefits of additional samples, getting written consent, and collecting the extra samples was time consuming; so in many cases, if midwives had a busy timetable, they would not offer the additional screening tests.

Recruitment took place in 16 health centres distributed across the region, and the number of women participating in this study varies greatly. But, between April and October 2001, the recruitment increased significantly due to a sub-study to evaluate VIA performance that was carried out with the aid of another midwife who took cervicophotographies on at least 100 women per health centre. One would expect that women will refuse screening and will only attend after being absolutely sure of the benefits of it, especially in an area where previously screening was so bad implemented. Women feared to be examined and not to receive feedback at all, as was the case for many years. But in this study, 35 women who were screened only with VIA and got the news from neighbours or friends about cervical additional tests that identified more positive women, came back and asked for another complete screening; furthermore, 19 women attended two health centres in different parts of the region just to be sure of their results.

Despite distance, most women who needed second samples, further evaluation or treatment did come back to the health centre or travelled to

the main hospital of their local area after being visited once by health workers. Transport costs were covered when required.

6.3. Health providers

One advantage of VIA is that it can be performed by a variety of health providers. VIA is easy to learn, and several studies have proved that good training and supervision would guarantee its accuracy.

VIA has been performed by cytotechnicians [138,193], clinicians [178,189], nurses [183,187,139,188], midwives [135] and medical doctors and nurses [192]. In the Zimbabwe study, women were interviewed and examined by a nurse-midwife; while in the Belinson study [172], gynaecologic oncologists were the examiners.

In Peru, midwifery and nursery are two different five-year-university careers; a university in Tarapoto offers both careers, and every year 15 midwives (men and women) are graduated. Midwives are better trained on female health, they know about sexually transmitted diseases and the reproductive system quite well, they are in charge of taking Pap smears when doctors are not available, so it was easier to train them to perform VIA; and it is more difficult to rely on doctors who are more expensive and in some places not available.

Our design required that if VIA was positive it ought to be confirmed by VIAM performed by a general doctor using and AviScope™. Midwives were then in charge of cervical samples collection (CC, LBC and HPV) and of VIA. Doctors performed VIAM and cryotherapy whenever appropriate.

Midwives and doctors were first trained during a four-day course, which included practice with silicon models and real patients. A team of five specialists trained participants during the course. Three gynaecologist oncologists with previous experience on VIA performed VIAs, VIAMs and cryotherapies, one gynaecologist oncologist did the colposcopies, LEEPs and cold cones with anaesthesia provided by the fifth specialist. The two

local gynaecologists previously trained in colposcopy at INEN, took part during colposcopies and surgery procedures.

After 3 months, trainees were reunited again, to report problems encountered in the field and to discuss difficult cases (or false negative cases detected by LBC). Afterwards, a gynaecologic oncologist visited the region to supervise the providers periodically.

When recruitment started, several doctors did not count with the AviScope™, so VIAMs were delayed. Problems with cryotherapy equipments were quite common, and solutions were not soon enough available, as a consequence, cryotherapy was almost never offered immediately.

Some of the first general doctors trained were in their "SERUM" year (one working year for the Ministry of Health in order to apply for residence programs), and so, they left the region within 6 months. All doctors and midwives took annual leave (one month) during the recruitment period, and moreover, some midwives also moved to different regions. Training of new providers had to be done regularly, and change of providers affected VIA and VIAM performances.

6.4. The information system

During the study, an information system for the TATI project (SYSTAT) was developed and used to maintain a database containing all information from collected data forms. A good screening programme should always have a reliable, up-to-date information system, which highlights the need for different referrals.

Despite the fact of counting with SYSTAT, information was not daily updated. Delays were caused because DIRES policy was to send information through the health network, unless extremely urgent, once per month. This translated into several problems.

For instance, women who knew they were positive in any screening test would appear in either of the colposcopy centres claiming to be examined

by an specialist before they data forms (requiring the exam) had arrive to the central TATI office in Tarapoto and so, before this information was inputted into SYSTAT. The results from LBC and HPV testing because of being processed in a parallel database arrived earlier to the health centre so midwives recalled women as soon as possible.

A number of women were screened twice in the same health centre or simply by two or three different health centres. As information was so delayed, the only way of identifying duplicates was using SYSTAT, which checked similar names before accepting a new screening record. However, there are a large number of women with identical names in the region.

SYSTAT also helped to identify women requiring colposcopy because of their additional samples results (once these were inputted into the system). It was also used to produce monthly reports of screening targets, number of women with disease, number of treated women, etc.

The use of an information system in any screening programme is crucial, but it should be accompanied but well-kept laboratory and clinical records from each laboratory or health facility involved in the programme. It should be updated regularly in order to give precise and opportunity information.

6.5. Screening tests

This section assesses advantages and problems of each technique, and evaluates positivity rates of each screening test. After discussing the histology results in section 6.5, sensitivities, specificities and PPVs of each test are presented, compared and discussed in section 6.6.

6.5.1. VIA

The main advantage of using VIA as a screening test in a low-resource setting like San Martin is the ability to give immediate results to women. VIA is a simple, easy and fast exam, which does not require the examiner to be a high-qualified doctor. We expected a positivity rate of 20% for VIA, and we got 24%. This result is similar to those of other studies

[180,187,172,188], in particular, to the recent study in India, which reported a 24.2% positivity rate of VIA for a low-threshold positivity and 15.8% when using a high-threshold criteria (the latter only included definite, well-defined acetowhite lesion in the squamocolumnar junction or close to the external os). In our study, midwives were taught to consider VIA positive if observed acetowhite lesions were close or in the squamocolumnar junction.

The positivity rate of VIA varied by health centres and by time of screening. Table 6.4.1.1 shows the number of women testing positive by month.

Most of the health centres finished recruitment for our study in September. During October and November, women were screened in H.R. San Jose de Sisa (in the centre of the region) and in the north. The last 200 women were screened during a special campaign in Tocache in December 2001.

Table 6.4.1.1. Positivity rate of VIA by month of screening.

	Women examined with VIA		
	No. VIA	No. women	Positivity
February	38	474	8
March	18	260	7
April	54	244	22.1
May	157	775	20.3
June	357	1026	34.8
July	329	1010	32.6
August	183	938	19.5
September	174	525	33.1
October	5	19	26.3
November	13	95	13.7
December	21	200	10.5

During the first two months of the project the overall positivity rate of VIA was very small (7.5%), it seems that midwives were just getting used to the technique and were not enough confident to identify possible cervical lesions. But as time went on, positivity rates increased significantly.

Between April and September, the positivity rate of VIA varied between 20% to 35% (Chi-square=102, df=5, $p<0.0001$). There is no straight forward explanation for this significant variation, but most probably is related to differences in midwives performance, and indeed to personal knowledge, abilities and commitment to the study.

Table 6.4.1.2 shows the number of women testing positive on VIA screened by each midwife before, during and after the cervicophotography sub-study. Unfortunately, data on midwives from H.R. Picota, H.R. Saposoa, H.R. Nueva Cajamarca, C.S. La Merced, C.S Nueva Rioja and P.S. San Juan del Rio Soritor are missing. However, based on available data, the positivity rate of VIA increased substantially while the cervicophotography sub-study was taking place, and decreased afterwards; except for the positivity rate of the midwife in P.S. Juan Guerra which continued increasing (up to 20%) after the sub-study ended.

One explanation for the increase in positivity rates during the sub-study could be that midwives feared to miss lesions that would appear obvious on the cervical photos. This suggestion highlights the weakness of VIA being a very subjective exam, the results depending entirely on the provider.

It is worth to mention that the H.R. Tocache only contributed to our comparative study with 400 women screened with VIA, LBC and HPV for the purposes of the cervicophotography sub-study during two attempts: one in May 2001 when the photos were damaged and one in December. When comparing both periods, the positivity rates of VIA were reduced from 32.7% (midwife 1) and 20.2% (midwife 2) in May to 11% and 10% in December, respectively.

Table 6.4.1.2. Positivity rate of VIA by midwife before, during and after Cervicophotography (CVP) sub-study.

Midwife	VIA positive women (Positivity rate %)		
	Before CVP	CVP	After CVP*
C.S. Lluyllucucha 1	39(25.5)	38(32.2)	
C.S. Lluyllucucha 2	25(16)	32(25.8)	
C.S Soritor	14(6.7)	21(16.5)	1(5.6)
C.S. Jepelacio	6(25)	64(63.4)	
Centro Materno Perinatal 1	4(13.3)	96(44.4)	35(37.6)
Centro Materno Perinatal 2	16(30.2)	108(52.7)	7(36.8)
P.S. Juan Guerra	3(18.8)	44(33.3)	7(41.2)
C.S. Tabalosos	16(16.3)	45(31.7)	16(31.4)
H.R. Lamas	25(29.4)	104(44.4)	1(4.2)
H.R. San Jose de Sisa	31(15.1)	74(52.9)	10(20)
H.R. Bellavista	7(8.1)	69(39.9)	22(24.7)

*Including only midwives with more than 15 VIAMs per period.

In a recent study in Nicaragua [192], 6 medical doctors and 14 nurses performed VIA in 1080 patients, obtaining a VIA positivity rate of 32.7% and that 88.2% of women with HSIL or worse lesions had a positive VIA. The authors found that the percentage of women with HSIL or worse lesions identified by VIA was increased after performing 100 or more examinations from 83% (5 to 99 examinations) to 92% (100 or more examinations) and conclude that performance of VIA increase with experience. They also compared the percentage of women testing positive on VIA who did not had confirmed HSIL after performing a minimum of 100 examinations obtained by doctors (77%) and nurses (75%), and found no statistical difference, but 83 women were classified as positive by doctors in comparison with only 16 women considered positive by nurses. Unfortunately, they do not report the positivity rates of VIA by health provider, but it appears as VIA positivity rates were higher among doctors.

There is a clear need for establishing uniform criteria on VIA positivity and to prepare standard training material for different type of providers. Currently, there are several on-going studies, which are trying to evaluate the performance of midwives who are full-time dedicated to screening; unfortunately, the results of these studies would not be comparable to ours, nor applicable to routine practice on low-resource settings, but they should contribute to clarify our findings in particular those related to differences of VIA performance among different providers.

6.5.2. VIAM

It is possible that the sensitivity of VIA performed by a health worker (nurse, midwife, cytoscreener) could be improved when having a second opinion of the test by another health worker; or having a second opinion by a doctor; or when incorporating the use of a magnification device, VIAM.

This is the third study where VIAM has been added to the screening scheme. Denny and colleagues, also implemented VIAM in both of their studies in South Africa [139,188], but their intention was to enhance the sensitivity of VIA, so VIAM was performed by the same nurse performing VIA. In their first study in Khayelitsha, they used a 2.5x magnification device (Edmund Scientific, NJ) and in the second one in Cape Town, an AviScope™ (4.5x magnification, PATH) similar to the one used in our study. In both studies they did not find a significant improvement in sensitivity of VIA when using a magnifying device.

In our study, VIAM was implemented:

1. to confirm the midwife VIA positive diagnosis;
2. to offer immediate treatment with cryotherapy if VIA positive diagnosis was confirmed.

During the training course, doctors did not find much advantage of using the AviScope™. Furthermore, in at least ten cases, they performed VIA instead of the midwife. After explaining the importance of following the protocol, this did not happen again. Doctors felt more comfortable

performing VIAM as the study went on. They only performed VIAM after a positive midwife diagnosis, which was confirmed in 534 women who were examined by a doctor using the AviScope™, reducing the referral rate in more than 50%.

With regard to offer immediate treatment to VIA positive women, in many instances, doctors were not available or did not count with the AviScope™ to perform VIAM or did not have the cryotherapy equipment or such equipment was not functioning properly at the time of treatment.

Only 329 women considered positive in VIAM were treated with cryotherapy, the other 205 were referred to colposcopy. Doctors were told to perform cryotherapy if the lesion was visible, not inside the endocervical canal and did not cover more than 75% of the cervix, but only 75 women who were referred to colposcopy had lesions with these characteristics, including those of 13 women referred directly to the colposcopist by the midwife. In some cases, doctors referred women to colposcopy because of their problems with cryotherapy equipment, but in general, it was hard for them to decide if cryotherapy was appropriate. Among 305 women positive on VIA_M treated with cryotherapy, 18 (6%) punch biopsies (taken before treatment) were inadequate (including one with pending result), 251 (82%) were negative, therefore overtreated, 18 (6%) were mild dysplasias and 18 (6%) were moderate dysplasias or worse lesions. Of 205 women referred to colposcopy, 11 (5%) had mild dysplasia and 39 (19%) had moderate dysplasia or worse lesions on histology. It seems that doctors used the wrong threshold: they either failed to take the biopsy of the right place or they were overcalling.

Despite the fact that there are a number of colposcopies not performed yet, and some pending histology results, VIAM reduced the referral rate of VIA substantially (more than 50%) but doctors have problems collecting biopsies and deciding between treating with cryotherapy or referring to colposcopy.

Other studies are needed to evaluate the use of a second opinion by a health worker and a confirmatory opinion by a doctor, and if either option is improved with the use of a magnification device.

6.5.3. Liquid-based cytology

This is the first time LBC is used in Peru. The option of using the AutoCyte-Prep® manual system (TriPath) was taken because Cytic, the manufacturer of ThinPrep®, refused to supply sampling and processing kits for our study. They decided not to participate in a study where a new ThinPrep® laboratory was to be implemented in South America, due to the costs of maintenance of equipment and quality assessment implied. On the other hand, the manual AutoCyte-Prep® system was more affordable for the laboratory (very few extra equipment from standard was needed), sampling and processing kits were cheaper and the Peruvian/South American representatives of TriPath, Capricorn Technologies Inc., offered training for the laboratory personnel.

The laboratory was then established within the Cytology Laboratory of INEN, and the training took place during three days in the beginning of March 2001. An expert with experience in retraining cytopathologists in the reading and interpretation of thin layer slides, carried out the training, which consisted of one morning of theoretical classes and two days of practice, both of the slides preparation (AutoCyte-Prep® procedures) and reading. The first two hundred and fifty collected samples from women screened in Tarapoto, were processed and the slides were read using a multihead microscope during the course. All participants, cytotechnicians and cytopathologists, found it difficult to read the very first slides and cytopathologists found it hard to agree with the expert diagnosis in the beginning, but after discussion they got into agreements. Once the training was finished, cytotechnicians from INEN carried out the processing of other 400 samples and mastered the technique. Afterwards, all LBC samples have been processed and first read by the technicians, and confirmed by the lead cytopathologist of the laboratory.

Several studies have agreed that correct interpretation of LBC slides improves with experience, after an initial period of acclimation. It is well-known that AutoCyte-Prep® slides have a cleaner background (less inflammation, less blood and debris), have less cells (even dispersal of cellular material) with less overlap and better nuclear preservation [156,155,157,158]; although an advantage, it also tends to generate initial overdiagnose after first training on reading LBC. And that, once cytotechnicians and cytopathologists become aware of their overdiagnosis, inversely, they start to underdiagnose [156,208,209].

In our study, the tendency to overdiagnose has been consistent. A quality assessment done after the first 1,000 samples were processed and read in the new LBC laboratory in Lima showed a 30% of overcalling. Seventy-nine LBC slides (of the first 1000) were reviewed by Dr. Vassilakos laboratory in Geneva. Table 6.4.3.1 presents a summary of the results.

Among 79 reviewed slides, both laboratories agreed in 53 (67%). The laboratory in Lima had an overcall rate of 30% (22/73). Only one LSIL was recognised by the Geneva laboratory, this was classified as HSIL by the laboratory in Lima. All HSIL or worse lesions were detected by LBC in Lima but one was classified as LSIL.

Table 6.4.3.1. Summary of results of quality assessment of LBC slides.

Experts from Geneva	Laboratory in Lima				
	Negative	ASCUS	LSIL	≥HSIL	Total
Unsatisfactory	5	0	1	0	6
Negative	43	7	8	1	59
ASCUS	0	2	3	2	7
LSIL	0	0	0	1	1
≥HSIL	0	0	1	5	6
Total	48	9	13	9	79

The ordered kappa statistic was 0.6156 (observed agreement=91%, expected agreement=76%, p<0.001) using weights: 1 if complete agreement, 0.889 if one category apart, 0.556 if two categories apart), and zero if three categories apart (complete disagreement).

Cytologists in Peru and Chile proposed the classification used to report LBC and CC results in this study, and all investigators supported it. As the purpose of the study was to detect high-grade lesions, in many instances results are merged together in less categories, but it has been difficult to compare results when LBC slides have been reviewed or have been correlated with histology. In the future, it will be better to use the Bethesda system, which is widely used internationally [13],[14].

The positivity rate of LBC for detecting LSIL or worse lesions (including ASCUS, AGUS and Condyloma/HPV) was 18%, when only mild dysplasias or worse lesions were considered, the rate was reduced to 9.6% and for detecting HSIL or worse lesions was 3.3% (171/5257). These are the highest positivity rates ever reported in Peru. So far, 105 women of these 171 with at least HSIL on LBC have been biopsied, the histology results are as follows: one inadequate, 29 negative, 12 condylomas/HPV, 12 mild dysplasias, 7 moderate dysplasias, 7 severe dysplasias, 22 carcinomas in situ, 3 microinvasive cancers and 12 invasive cancers, confirming 23% of LSIL and 49% of HSIL or worse, and that 28% were wrongly classified as HSIL. These figures are far from those of Vassilakos laboratory [144], where among 357 patients classified as having HSIL or worse lesions, 89.6% were histologically confirmed, 5.6% were LSIL and 4.5% were negative on histology.

Several studies have pointed out that the number of inadequate samples is reduced when using liquid-based cytology methods [145,146,209,147,148,149,150,151,152,153,159]. Because samples are collected using a brush, which head is removed from its handle and immersed directly into a preservative vial, the smear-taker is not any more in charge of fixation, and so cells are unlikely to be assessed as inadequate, equivocal or borderline because of poor fixation [155]. The percentage of inadequate samples in our

study was 5%, most of them were not possible to evaluate because of haemorrhage (195 cases) or for being hipocellular (44) or acellular (4). One reason for so many cases of haemorrhage could be the way of sampling, the Rovers-Cervex® brush was rotated five times clockwise once inserted into the endocervical canal, this was an entirely new procedure for midwives who claimed that most screened women had severe cervical inflammation at the time of screening. The LBC laboratory reported 26% of slides with inflammation, and 0.5% of them with severe inflammation. As for infections, 519 (10%) women had an infection on LBC, 390 (7%) had gardnerella, 65 (1.2%) had candidiasis, 63 (1.1%) had trichomonas, two had herpes virus, one had chlamydia, and two had two infections: one chlamydia and trichomonas, and one had gardnerella and trichomonas. These are also the first figures regarding inflammation and infections detected on cytology.

As compared with conventional cytology in this study and previously in Peru, this is the first report of a 3.3% positivity rate of cytology for detecting high-grade disease in a Peruvian population, the first time the percentage of inadequates is below 10%.

LBC collected samples were sometimes transported over 30°C of temperature, but were still satisfactory for evaluation. The majority of inadequate samples were related to bleeding processes while collecting samples. The Rovers-Cervex® brush may be too hard for the cervix, especially, if this is inflammated, and definitively, rotating it five times, produces bleeding in most cases.

It is clear that LBC has had more than 25% of overcalling, but further quality assessments are needed to estimate real values of overdiagnosis and underdiagnosis. However, LBC detected 51 (1%) high-grade lesions, including 12 invasive cancers.

It has been suggested that LBC slides are prepared more quickly than conventional cytology ones and so that laboratory productivity can increase 25-30% [159]. This has not been evaluated properly yet, and one has to consider that despite the fact that several LBC slides are produced at

once, the slide preparation is more complex and takes longer time than a simple Pap smear [154].

In Peru, it is not of great concern if productivity is enlarged by the use of LBC. If LBC were to be used greatly in Peru, two to three laboratories located in key localities in the country would have to be established and would be regulated by a central one in Lima. Currently, there are many conventional cytology laboratories in Peru that lack quality standards, for instance, the amount of slides read per day greatly exceeds the limit established in the UK (32 per day) and the USA (100).

In summary, further quality assessments have been planned to evaluate if the LBC laboratory has improved its performance with experience. The first results of LBC are so far very promising when compared with conventional cytology, but LBC laboratories need training and continuing education, in order to achieve quality standards and these can become very costly.

6.5.4. Conventional cytology

Conventional cytology was not considered as a screening option, because after being in place for more than 20 years, it showed no benefit for the health of women in San Martin. Conventional cytology has many problems in the region: often negative women are re-screened several times so the number of previously unscreened women covered is minimum. Sample collection is of bad quality, and samples can get easily lost or broken in their way to the laboratory. The local laboratory does not have internal or external quality assurance protocols, processing and reading of PAP smears is below quality standards. The results are not given to women unless they specifically asked for them, and if they are positive, no further evaluation or treatment is offered to them, and women have to arrange private health care if they can afford it.

A clear example of the conventional cytology situation in the region was shown during the training of midwives on VIA and general doctors on VIAM and cryotherapy in November 2000. A special effort was made to

identify and visited 233 women with previous positive conventional cytology (LSIL or worse during 1999 and 2000) [9]. These women were invited to participate in a special screening and treatment campaign, and those who agreed to participate were transported to Tarapoto, where they were examined by the trainers and treated as required. Before this training course, none of these women had been recalled for further evaluation or adequate treatment, and at least 10 of them had carcinoma in situ or invasive carcinoma cytology results. All these women have been treated as part of the TATI project.

Several attempts to improve conventional cytology in the region since the start of the TATI project have been carried out. Sponsored by the project, cytotechnicians have been retrained at INEN and have undergone proficiency tests. The problem of inadequate sampling has also been addressed; midwives with the smallest inadequate rates have standardised the collecting procedure and have shown it to each midwife in routine practice. The central laboratory in Tarapoto did not have an appropriate storage system of slides, the TATI project have provided with infrastructure to maintain slides and for keeping records.

6.5.5. HPV testing

It is a fact that HPV testing has a role in cervical screening [210,211]. Several studies have shown the advantages of using HPV testing as a screening technique, but it is not been well established which role HPV testing could take. The most important one would be to consider HPV as an adjunctive to either LBC or VIA. In this study we tried to prove that HPV could be an adjunctive test to VIA, women testing positive on VIA could be sampled for HPV and if positive then proceed to further evaluation or treatment. We only tested the samples collected sometime later so the assessment of women having high-grade disease was not based on HC-II. HPV samples were tested in London but ideally testing should have been carried out in

Lima. The possibility of establishing a Hybrid Capture Laboratory in Lima was not feasible at the time of the study but it is now being implemented.

This is the first time that HPV collected samples are used in a very difficult setting. Efforts were made to keep samples under 30°C, but sometimes cars transporting them were trapped in dirty roads for many hours and collected HPV samples were exposed to temperatures over 35°C.

HPV samples were collected in third place after conventional and liquid-based cytology, and this did not affect its positivity rate.

The overall prevalence of HPV infection in this study was 13%, similar to that of other studies. The prevalence of HPV infection was 20% in a previous cross-sectional study in asymptomatic women in a deprived area from Lima using PCR to detect HPV 6, HPV 11, HPV 16, HPV 18, HPV 31, HPV 33 and HPV 35; after excluding women positive for HPV 6 and HPV 11, the prevalence was reduced to 10.4% (unpublished data). In a hospital-based case-control study in Lima [5], the prevalence of HPV infection among healthy controls was 18%, once more, after excluding low-risk HPV types; the prevalence was reduced to 13%. In a systematic review of HPV testing, Cuzick *et al* [169] obtained an overall positivity rate of tests for high-risk HPV types (using PCR or Hybrid Capture) of 13% ranging from 10% for PCR using GP5/6 primers to 20% using HC-II, in asymptomatic populations.

Because of these consistent results, we can assume that the positivity rate of HC-II was not affected by high temperatures in the field or by the order in which samples were collected. Furthermore, there was only one insufficient sample for testing. This was significantly impressive if compared with inadequate rates observed for both cytology techniques (5% and 11% for LBC and CC, respectively).

HPV prevalence decreased, as women grew older, as shown in Table 5.2.1.3 (p-value for trend<0.001). We screened women between 25 and 49 years of age, however, 61 women younger than 25 years of age and 12 older than 49 participated in the study.

Table 6.5.5.1 presents the age distribution of HPV positive women. As mentioned in 5.2.1, when excluding those there is a decreasing trend prevalence of HPV decreased with age, from 15% in women 25-29 years to 9% in those aged 45-49.

HPV prevalence varied between 7% (only 165 women were screened) and 15% across health centres of recruitment (Table 5.2.2.3). After excluding the C.S Juan Guerra where only 165 women were screened (positivity rate of HC-II of 7%), the prevalence of HPV infection was consistent among health centres (mostly between 11% and 14%, p-value=0.249).

Overall, we considered positive women with a viral load of at least 1 RLU, but the prevalence of HPV infection was 10% when instead a high threshold (≥4 RLU) was used.

Table 6.5.5.2 presents the distribution of HPV results (RLU) obtained in our study, and the positivity rate of second HPV testing by RLU of initial testing.

Only 82 women (1.6%) had borderline HPV results (0.8-2 RLUs). Among 702 positive women (≥1 RLU), 10% had less than 2 RLUs, 10% between 2 and 4 RLUs, 15% between 4 and 10 RLUs, 35% between 10 and 100 RLUs and 30% over 100 RLUs.

It has been suggested that viral load predicts persistence of HPV infection [81,89]. In our study, we were not able to assess this relationship, because we used HC-II, and according to a recent paper by Gravitt *et al* [212], RLUs do not predict well viral load. However, it is worth to mention, at least 50% of women who had 4 RLUs or more on initial HPV testing had a second positive HPV test.

In summary, the prevalence of HPV infection was 13% using Hybrid Capture II; which proved to be enough robust to be used in tropical conditions, had consistent results across health centres, and reported very few borderline results. It is worth noting that HPV testing was the only screening test not carried out in Peru; moreover it was performed in an international well-recognised HC-II laboratory.

Table 6.5.5.2. HPV viral load distribution and positivity rate of second HPV testing.

Viral load	n	%	% in positives	Second HPV testing No. positive/No. tested (%)
<0.8	4821	86.9		23/300 (8)
0.8-1	23	0.4		1/4 (25)
1-2	69	1.2	9.8	10/31 (32)
2-4	65	1.2	9.3	8/23 (35)
4-10	104	1.9	14.8	21/42 (50)
10-100	248	4.5	35.3	49/94 (52)
>100	216	3.9	30.8	33 /65 (51)
Total	5546	100	100	145/559 (26)

6.6. Histology

Punch biopsies were used before treating positive VIA_M women with cryotherapy. General doctors took biopsies using only the AviScope™ (not during colposcopy), therefore, they had difficulties in collecting a good sample. In addition, because women tended to bleed, treatment was frequently delayed or women were referred to colposcopy instead.

Local gynaecologists were trained during one or three months to perform colposcopy and to treat with LEEP at INEN in Lima. In the beginning of the study, colposcopists were not enough confident to collect biopsies of apparent lesions. In many cases, colposcopies were repeated even three times before a biopsy was collected or a woman was treated.

Table 6.6.1 shows the preliminary results of a quality assessment of histology performed in the UK. The results were as follows:

Table 6.6.1. Summary of results of quality assessment of histology.

Expert in UK	Laboratory in Lima				
	Unsatisfactory	Negative	LSIL	HSIL	Total
Unsatisfactory	8	23	2	1	34
Negative	1	51	55	4	111
LSIL	0	3	8	7	18
HSIL	0	0	1	17	18
Total	9	77	66	29	181

A total of 274 pathology slides were reviewed corresponding of collected biopsies from 181 women. This review is still on going; some sets of pathology slides from a biopsy are still to be reviewed. Therefore, it is possible that some results coded so far as unsatisfactory change once the corresponding set of slides is complete.

Both laboratories agreed in 84 (46%) pathology results. The laboratory in Lima had an overcall rate of 45% (66/146). Only 18 LSIL were recognised by the UK laboratory, 3 of them were classified as negative by the laboratory in Lima and 7 as HSIL. Seventeen of the 18 HSIL (or worse lesions) were diagnosed as HSIL by the laboratory in Lima; only one was classified as LSIL.

The ordered kappa statistic was 0.4218 (observed agreement=79%, expected agreement=64%, $p<0.001$) using weights: 1 if complete agreement, 0.889 if negative in one laboratory and unsatisfactory in the other, and 0.556 if unsatisfactory in one laboratory and LSIL in the other or one category apart), and zero if unsatisfactory or negative in one laboratory and HSIL in the other (complete disagreement).

The level of disagreement is highly related to the number of unsatisfactory samples, the reviewer considered unsatisfactory or non-contributory samples:

- Those who had only endocervical tissue, failing to identify the part of the cervix where the SIL might have been expected and from where the biopsy should have been taken; and,
- Those obtained from LEEPs, where the surface epithelium was completely destroyed.

Of special concern are four biopsies considered negative by the reviewer but HSIL by the original pathologist; three of them had an additional slide still to be reviewed, the other has either been overdiagnosed by the laboratory in Lima or misclassified by the reviewer; a third reading is being arranged.

As stated before, this review is yet to be continued, however, it could be recommended that more training and supervision of biopsy collection and treatment of local specialists of the region of San Martin should be encouraged. Regarding results, the likelihood of collecting biopsies from the wrong parts of the cervix might have diminished our rate of high-grade disease, and somehow enlarged the false positive rates of our screening tests. Nevertheless, the effect on tests performance would be the same for all screening techniques.

6.7. Statistical methods to estimate measures of performance of screening tests

At time of analysis, the data was incomplete. We used a very complicated analysis, in order to provide good estimates of sensitivity, specificity and positive predictive value for each screening test and for several combinations of them. Data will most probably be complete in six months.

One main problem of having done this estimation before data completion relies in the fact that it is almost impossible that HPV positive women who were LBC negative and VIA_M negative have had confirmed HSIL (as these women would have not been fully evaluated yet). More generally, the likelihood of definite disease status (HSIL or no HSIL) is heavily depending on screening tests scheme.

Whereas other similar studies have used a direct calculation of the proportion of evaluated women and applied it to the general group; in addition to the proportion of women not fully evaluated within groups, we had a very large proportion of women with missing VIAM or second screening results. There may be other ways to handle these difficulties, but we decided to estimate the distribution of the missing values and used for estimation.

The algorithm used to estimate sensitivity, specificity and positive predictive values yielded unbiased estimators, but we needed to use bootstrapping to calculate confidence intervals.

6.8. Measures of performance of screening tests

HPV testing (HC-II) had the highest sensitivity of the screening tests (72%, CI: 65,79), but moderate specificity (90%, CI: 89,91) for detecting high-grade disease. LBC (considered positive if ASCUS or worse lesions were diagnosed) had the second highest sensitivity (69%, CI: 61,78) and lower specificity (85%, CI: 84,86) than HPV testing. The most unexpected results were those of VIA which had an extremely low sensitivity (44%, CI: 34,59) and a poor specificity (77%, CI: 74,77). Combining VIA/VIAM, reduced the sensitivity to 31% but increase the specificity to 91% (CI: 91,92).

Overall, these results are very disappointed as compared with other of similar studies in the literature.

The sensitivity of HPV testing (using HC-II) has been consistently reported over 80% [75,173,171,170] and over 95% in more recent studies [174,172,161]. HC-II missed 26 high-grade lesions: zero cancers, 3 carcinomas in situ (in women aged 26, 35, and 43), 4 severe dysplasias (in women aged 28, 29, 39, 45) and 19 moderate dysplasias. Of these 26 lesions missed, 6 were detected by VIA and LBC, 14 were identified only by VIA, 5 only by LBC, and one was detected during a non-indicated second screening. In contrast, HC-II specificity was higher than that previously reported by other studies except for the Cuzick study [75] in women older than 35 years of age. It is clear that

if moderate dysplasia were excluded from our definition of HSIL, the sensitivity of HC-II would be much better. It remains to see how many of the missed cases of HSIL are recoded to LSIL on review by the expert pathologist.

The sensitivity of conventional cytology has been reported to vary between 40% and 80% [134,138,135,139]. Belinson *et al* [172] reported a sensitivity of LBC of 77% using ThinPrep at a high threshold (≥HSIL), and Vassilakos and colleagues [142] a sensitivity of 99% using AutoCyte-Prep® (low threshold ≥ ASCUS). In our study, the sensitivity of LBC using AutoCyte-Prep® at a low threshold was not as high as that from Vassilakos group, but at least was higher than 65%. LBC was inadequate in one microinvasive and two invasive cancers and negative in other 27 HSIL lesions (including one invasive adenocarcinoma); 9 of these lesions were detected by VIA and HC-II; 14 were identified only by VIA, 3 only by HC-II, and one during a non-indicated second screening. The three inadequate LBC samples that resulted in cancers were detected by HPV, and only the microinvasive cancer was missed by VIA. It is worth mentioning that conventional cytology (CC ≥ASCUS) only considered positive 30 of 75 (40%) high-grade lesions detected by LBC. But CC also identified two of the cancers whose LBC samples were inadequate and another high-grade lesion missed by LBC.

The specificity of LBC was low; this is not surprising, as it was cleared throughout the study that LBC was overcalling lesions regularly.

The most striking results as those of VIA (and combined VIA/VIAM). This study was designed based in the assumption that VIA would have a high sensitivity but poor specificity as shown in several other studies, and that HPV testing or LBC could be used as an adjunctive screening test to improve low specificity. But VIA (and combined VIA/VIAM) had the lowest sensitivity of all screening tests. VIA missed 23 high-grade lesions, 3 microinvasive cancers and 10 invasive cancers (including one adenocarcinoma); 27 of which were identified by both LBC and HPV testing, 5 only by LBC and 2 only by HC-II, and one during a non-

indicated second screening. However, VIA was able to identify 25 and 20 additional cases missed by LBC and HPV testing, respectively.

As expected combinations of VIA with other screening tests improved VIA specificity with a loss in the already so poor sensitivity.

The highest positive predictive values was that of LBC (≥HSIL) had the highest positive predictive value (44%, CI: 34,53), followed by HC-II (22%, CI: 14,32).

Overall, HC-II did not miss any invasive carcinoma; LBC missed one invasive adenocarcinoma and was unable to identify three cancers because cervical samples were inadequate; and VIA was unable to identify 10 of 18 detected cancers (56%).

Glandular abnormalities are not readily detected by cytology [213,214], but it is accepted that invasive and preinvasive lesions would be found in 40% and 20-28% of abnormal glandular smears [215,216]. Being the LBC laboratory recently established, it is not surprising that it failed to identify one invasive adenocarcinoma.

A critical case is that of VIA, it is unacceptable that it missed 10 invasive carcinomas. One possible explanation but not justification for such failure could lie on the fact that women coming into screening frequently suffered of severe cervicitis, and this must have hide lesions even after acetic acid was applied. Nevertheless, we are in the process of reviewing the clinical data forms, investigating the stage of these cancers, and arranging the correspondent histology review.

In summary, HPV testing had higher sensitivity than all the other screening tests, LBC had moderately low sensitivity and specificity but its results were improved as the new LBC laboratory gained experience. VIA had very poor sensitivity and specificity, but was able to identify additional high-grade lesions missed by either HC-II or LBC. This is the first screening study within an organised intervention to be reported for Peru, in any case, the number of preinvasive lesions and invasive carcinomas

identified by each screening test suggest that each of them is better than previous opportunistic cytology.

6.9. Future of cervical screening in Peru

Given the results of this study, it is not clear yet which should be the best approach to cervical screening in Peru.

The lack of facilities in different parts of the country as in the San Martin region makes it very difficult to establish a well-organised programme. The need for decentralization has become obvious after this study, without a pathology laboratory, without well-trained colposcopists and gynaecologists capable of treating high-grade lesions, any effort to screen is useless, since it is critical to treat all lesions found and this, has not happened in Peru for decades.

Different approaches would need to be put in practice depending of the screening target population and the health facilities available for such population.

Despite the poor results of VIA, the advantages of this test over opportunistic cytology are clear. In rural areas of Peru, the modality of health campaigns is very common: a group of health providers go into one locality and apply a complete basic health package which includes vaccination, screening, reproductive health advice counsel and immediate treatment of minor diseases. VIA can easily be used within health campaigns; a team consisting of a midwife, one doctor with cryotherapy equipment and one gynaecologist with a colposcope could travel to different areas, screen and treat (if appropriate). This scheme has already been in place within the TATI project and has allowed the screening of women living in very remote areas.

The possibility of using VIA as a primary cervical screening method is promising; but first, definition standards of VIA positivity, and well-documented training material should be developed and used to train VIA

providers. Because midwifery is offer in most universities in Peru, VIA could be taught initially at undergraduate levels and midwives could be retrained once in clinical practice. In this way, better and more consistent performance of VIA could be ensured; with no extra cost.

For urban areas, more sophisticated schemes can be applied where the use of HPV testing should be the first choice. Cuzick [217] have recently proposed that HPV could be used as a primary screening test, although this approach needs more evaluation, our results in very difficult conditions support this suggestion, and it would be expected that HPV testing would perform significantly better in less difficult conditions. However, ways of dealing with the anxiety to be generated in women testing positive on HPV should be proposed, discussed and normalised.

Another main problem is the fact that even in areas where health networks work quite well, information is not given the relevance it should, causing delays in the clinical management of positive women. Efforts need to be made in order to ensure that positive women receive adequate treatment within a reasonable time after diagnosis; and the first step to do so, is by ensuring that precise information is available when necessary; without trespassing ethical issues of anonymous records.

6.10. Screening developments

A number of new screening techniques for cervical cancer are currently under investigation.

Visual inspection after the application of Lugol's Iodine (VILI) is a promising one. In a study of 4444 women in India [193], the sensitivity of VILI to detect high-grade lesions was 87% and its specificity 85%, while those of VIA were 89% and 78% (for a high-threshold positivity definition).

Several studies have described biomarkers to be used in the detection of cervical neoplasia; of special interest are those related to the detection of p16 overexpression in HPV-related lesions [218]. HPV types have been subdivided into high- and low-risk categories based on their association

with invasive cervical carcinoma. This association is as well, based on the relative affinity that the HPV-type specific oncoproteins E6 and E7 bind to cellular regulatory proteins, specifically, the p53 tumour suppressor protein (Rb). Inactivation of these factors leads to disruption of the cell cycle and to overexpression of p16 levels in HPV-related lesions. High degree of correlation between the immunohistochemical detection of p16 in tissue sections and the presence of oncogenic-risk HPV-induced SIL lesions have been reported when using antibodies to p16 [219,220].

Cervical cancer and CIN express high levels of the cyclin-dependent kinase inhibitor p16, suggesting that staining for this marker could help to more precisely identify CIN in tissue sections and therefore reduce variation in interpretation of cervical lesions. Klaes R *et al* [221] stained biopsy sections with hematoxylin and eosin, and with a p16 -specific monoclonal antibody; and obtained 91% agreement in the interpretation of p16 expression between five experienced pathologists; p16 was expressed in CIN 2, CIN 3, and CIN 1 associated with HPV or cervical cancer. Even small CIN or cervical cancer lesions in biopsy sections were identified after p16 immunostaining. The authors concluded that p16 immunohistochemistry could reduce false-negative and false-positive biopsy interpretation.

In a more recent study, Agoff and colleagues [222] performed immunohistochemistry for $p16^{INK4a}$ and Ki-67 on 569 and 432 biopsy samples, respectively. The degree of $p16^{INK4a}$ and Ki-67 expression correlated with degree of cervical neoplasia, and $p16^{INK4a}$ was less likely to be positive in samples from women with negative, reactive, and atypical biopsies. The authors conclude once more that of $p16^{INK4a}$ has potential as a screening marker for cervical neoplasia. The use of a p16 immunohistochemistry test; which can give results within the same day of screening or even as a deep-stick test could change the future of cervical screening.

Although screening studies using $p16^{INK4a}$ need to be carried out, and more studies should evaluate the performance of VILI; the use of $p16^{INK4a}$ and VILI appear as promising cervical screening alternatives.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusions

In summary, the conclusions of this thesis are:

1. VIA is a cheap, easy screening test that gives immediate results, and can be easily implemented in routine midwifery clinics. VIA performance improves with experience and requires periodic retraining. Standard definitions of positivity and measures of proficiency on VIA and a minimum number of VIAs performed per week should be established. Well-documented manuals and updated literature on the technique should be available for VIA providers. With such quality assurance measures in place, VIA should provide a sensitive, but non-specific test for HSIL. However, in this study, the lack of supervision resulted in poor sensitivity emphasising the difficulties that must be overcome if VIA is to be used in mass screening programmes in developing countries.

2. VIAM reduced VIA referral rate in more than 50%. However the improved specificity was associated with a reduced sensitivity (from 44% to 31%). Additionally, doctors were not confident enough to discriminate between women suitable for immediate treatment and those needing colposcopy.

3. The advantage of VIA is that the result is available immediately. Hence, if screening is properly organised it should be possible to treat women at the same visit thus eliminating dropout associated with recall for treatment. The disadvantage is that its poor specificity would result in over-treatment and without adequate post-treatment surveillance, recurrence is likely [223],[224]. For instance, in a study by Benedet *et al* [223], among 1675 patients eligible for assessment at one year after being treated with cryotherapy, 6% had recurrence for CIN and 5.6% were lost to follow-up. Soutter *et al* [224], using data from four UK centres, reported 2.6% annual recurrence after treating CIN

with laser vaporisation, cold coagulation, laser cone or loop diathermy. Currently, it is not possible to carry out cytology or HPV testing during a single visit.

4. HC-II and AutoCyte-Prep® samples appeared to be robust to the high temperature of San Martin; and can be used in difficult resource settings even if not possible to comply with manufacturer's recommendations for storage.

5. The percentage of inadequate LBC samples was high but half that of conventional cytology. The positivity rates of LBC for low and high threshold of disease, and those of CC are the highest ever reported in Peru. LBC detected biopsy confirmed HSIL cases missed by VIA and VIA_M; and detected twice the number of histologically confirmed HSIL or worse lesions detected by CC.

6. HC-II is an objective test, had the highest sensitivity of all screening tests used in this study, and proved to be robust enough to be used in tropical conditions. But it is still significantly more expensive than VIA. However, if HPV testing could guarantee longer screening intervals, its use could be certainly justified, as the increase in cost will reduce the cost of several visits and of false positive referrals. HC-II would definitively have a place in organised cervical screening programmes in less difficult conditions within developing countries.

7. More studies using existing infrastructure, but with organised quality assurance should be carried out to evaluate the performance of VIA in routine clinical practice.

7.2. Recommendations:

1. Efforts should be made to train midwives on VIA while they are at undergraduate level.

2. Midwives should performed certain number of VIA examinations, receive regular training and have proficiency tests, in order to ensure better performance.

3. Better effort should be made to guarantee the performance of cryotherapy equipment before implementing the treatment in a large scale.

4. Training of colposcopists should be done under a standard curriculum and regular test of proficiency should be established.

5. The ideal "single-visit" scheme is not feasible in settings where qualified specialists are not available. Using external gynaecologists and other high-qualified health professionals is not practical in low-resource settings. Instead, a combination of VIA with another screening test such as LBC or HPV testing should be considered for low-resource areas; while HPV testing in combination with LBC is possibly more appropriate for areas with better facilities. The use of "health campaigns" both to screen and treat women once laboratory results are available (if applicable) is strongly recommended.

6. Decentralization of health resources is recommended before establishing a mass screening programme. Nevertheless, different approaches should be implemented depending on target population characteristics and health services available.

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APPENDIX:

Code used to calculate sensitivity, specificity and PPV after estimating screening tests results and disease status in women with imcomplete screening or women not fully evaluated.

```
prog def cal_eff, rclass

version 8.0

xi:mlogit viam1 i.lbc7 if via1==1
predict fitv0 fitv1 fitv2


expand 3 if viam1==. & via1==1 & eval==0
sort codigomu
gen pviam1=viam1
qui by codigomu:replace pviam1=_n-1 if pviam1==. & via1==1 & eval==0
gen wviam1=1
replace wviam1=fitv0 if pviam1==0 & viam1==.
replace wviam1=fitv1 if pviam1==1 & viam1==.
replace wviam1=fitv2 if pviam1==2 & viam1==.

lab var pviam1 "New VIAM after estimating VIAM pending result"
lab def pviam1 0 Neg 1 Cryo 2 Colp

/*New management groups after estimating VIAM pending*/

/*Combined results from first samples VIA and VIAM (pviam)*/
drop via_m1
gen via_m1=0 if via1==0 | (via1==1 & pviam1==0)
replace via_m1=1 if pviam1==1
replace via_m1=2 if pviam1==2
lab drop via_m1
lab var via_m1 "VIA_M"
lab def via_m1 0 Neg 1 Cryo 2 Colp
lab val via_m1 via_m1

drop via_mm1
gen via_mm1=0 if via1==0
replace via_mm1=1 if via1==1 & pviam1==0
replace via_mm1=2 if via1==1 & pviam1==1
replace via_mm1=3 if via1==1 & pviam1==2
lab drop via_mm1
lab var via_mm1 "VIA_M"
lab def via_mm1 0 "VIA neg" 1 "VIAM neg" 2 Cryo 3 Colp
```

```
lab val via_mm1 via_mm1


capture drop via_lbhg
gen via_lbhg=0
replace via_lbhg=1 if via_m1~=0 & lbchsil==1

capture drop vialbhg
gen vialbhg=0
replace vialbhg=1 if via1==1 & lbchsil==1

capture drop vialblo
gen vialblo=0
replace vialblo=1 if via1==1 & lbclow==1

capture drop viahpv
gen viahpv=0
replace viahpv=1 if via1==1 & hpv1==1

capture drop via_lblo
gen via_lblo=0
replace via_lblo=1 if via_m1~=0 & lbclow==1

capture drop via_lbhs
gen via_lbhs=0
replace via_lbhs=1 if via_m1~=0 & (dxlbc==8 | dxlbc==9)

capture drop via_lbca
gen via_lbca=0
replace via_lbca=1 if via_m1~=0 & dxlbc==10

capture drop via_cchg
gen via_cchg=0
replace via_cchg=1 if via_m1~=0 & cchsil==1

capture drop via_hpv
gen via_hpv=0
replace via_hpv=1 if via_m1~=0 & hpv1==1

capture drop lbhg_hpv
gen lbhg_hpv=0
replace lbhg_hpv=1 if lbchsil==1 & hpv1==1

capture drop cch_o_n
gen cch_o_n=0
replace cch_o_n=1 if cchsil==1 & lbchsil~=1 & hpv1~=1 & via_m1~=1
lab var cch_o_n "Only CC HSIL, others neg"

capture drop cch_o_lb
gen cch_o_lb=0
```

```
replace cch_o_lb=1 if cchsil==1 & lbchsil~=1
lab var cch_o_lb "CC HSIL & LBC<HSIL, others not consid"

capture drop hpv1020
gen hpv1020=0
replace hpv1020=1 if hpv1==0 & hpv2==0

capture drop hpv1021
gen hpv1021=0
replace hpv1021=1 if hpv1==0 & hpv2==1

capture drop hpv1120
gen hpv1120=0
replace hpv1120=1 if hpv1==1 & hpv2==0

capture drop hpv1121
gen hpv1121=0
replace hpv1121=1 if hpv1==1 & hpv2==1



capture drop rescreen
gen rescreen=0
replace rescreen=1 if (via_m1==0) & ((dxlbc>=4 & dxlbc<8)| ((dxlbc<4| dxlbc>90) &
dxhpv==1)) & (dxpap<8 | dxpap==98 | dxpap==99 | dxpap==.)
lab var rescreen "Need rescreening"

capture drop norescr
gen norescr=1-rescreen
lab var norescr "Not need rescreening"


xi:mlogit dxscreen i.lbcres i.hpv1 if rescreen==1 & via_m2~=. & lbc2~=. & hpv2~=.,
basecategory(0)
predict fits0 fits1 fits2

sort codigomu
gen codi2=_n
expand 3 if dxscreen==. & rescreen==1 & eval==0
sort codi2
gen prescr=dxscreen
qui by codi2:replace prescr=_n-1 if prescr==. & rescreen==1 & eval==0
gen wrescr=1
replace wrescr=fits0 if prescr==0 & dxscreen==. & rescreen==1
replace wrescr=fits1 if prescr==1 & dxscreen==. & rescreen==1
replace wrescr=fits2 if prescr==2 & dxscreen==. & rescreen==1

gen finwe=wviam1*wrescr
```

```
local clgp6 "via_m2==0 & ((foldxlbc>=1 & foldxlbc<=3 & foldxhpv==0) |
(foldxlbc==99 & foldxhpv==0) | (foldxlbc>=2 & foldxlbc<=3 & foldxhpv>90 &
foldxhpv<100))"
local clgp5 "via_m2==2 | (((foldxlbc>7 & foldxlbc<20)| foldxhpv==1) &
clingroup==.)"


/*Clinical management groups*/
drop clingroup
gen clingroup=1 if via_m1==1
replace clingroup=2 if via_m1==2
replace clingroup=3 if (via_m1==0 | viaviam==9) & ((dxlbc>7 & dxlbc<20) |
(dxpap>7 & dxpap<20))
replace clingroup=6 if via_m1==0 & (dxpap<8 | dxpap==98 | dxpap==99 | dxpap==.)
& ((dxlbc>=1 & dxlbc<=3 & dxhpv==0) | (dxlbc==99 & dxhpv==0) | (dxlbc>=2 &
dxlbc<=3 & dxhpv>90))
replace clingroup=4 if clingroup==. & prescr==1
replace clingroup=5 if clingroup==. & prescr==2
replace clingroup=6 if clingroup==. & prescr==0

replace clingroup=10 if eval==1 & clingroup==.

lab var clingroup "Clinical management groups"
lab drop clingroup
lab def clingroup 1 Cryo 2 Colp 3 "HSIL Colp" 4 "Cryo aft 2nd sa" 5 "Colp aft 2nd sa"
6 Neg 10"Full eval/incomp screen"
lab val clingroup clingroup


egen n_cg=sum(finwe), by(clingroup)
egen n_eval=sum(finwe*eval), by(clingroup)
gen ipeval=n_cg/n_eval

svyset[pw=ipeval]

sort codigomu
gen codi3=_n
expand 2 if eval~=1
sort codi3
gen hg=hgsil
qui by codi3:replace hg=_n-1 if eval==0
gen wei=eval
gen nohg=1-hg

egen fit3=mean(hgsil), by(via_mm1 lbc6 hpv1)
egen fit30=mean(hgsil) if lbc6==4, by(lbc6)
replace fit3=fit30 if fit3==.

gen wei3=1
replace wei3=fit3 if eval==0 & hg==1
```

```
replace wei3=(1-fit3) if eval==0 & hg==0

gen peso3=finwe*wei3


xi:svylogit hgsil i.via_mm1 i.cch_o_lb i.lbc6 i.hpv1 i.via_lbhg i.via_hpv i.lbhg_hpv,
subpop(norescr)
predict fitnor if norescr==1
count if fitnor==.
egen fitnor0=mean(hgsil), by (clingroup via_m1)
replace fitnor=fitnor0 if fitnor==.


xi:svylogit hgsil i.hpv2, subpop(rescreen)
predict fitres if rescreen==1
count if fitres==.
egen fitres0=mean(hgsil), by (clingroup via_m1)
replace fitres=fitres0 if fitres==.

gen fitfin=cond(norescr==1, fitnor, fitres)

replace wei=fitfin if eval==0 & hg==1
replace wei=(1-fitfin) if eval==0 & hg==0

gen peso1=finwe*wei

gen wei2=eval
xi:svylogit hgsil i.via_mm1 i.cch_o_lb i.lbc6 i.hpv1 i.via_lbhg i.via_hpv i.lbhg_hpv
predict fit
count if fit==.
egen fit0=mean(hgsil), by (clingroup via1)
replace fit=fit0 if fit==.

replace wei2=fit if eval==0 & hg==1
replace wei2=(1-fit) if eval==0 & hg==0


gen peso2=finwe*wei2


global rlist ""

foreach i of numlist 1/3 {
  sum hg [iw=peso`i'], mean
  scalar nhg=r(mean)*r(sum_w)
  sum nohg [iw=peso`i'], mean
  scalar nonhg=r(mean)*r(sum_w)
  foreach test in via1 viamcom lbclow lbchsil hpv1 vialbhg vialblo viahpv{
    sum `test' [iw=peso`i'] if hg==1, mean
    scalar se`i'_`test'=(r(mean)*r(sum_w))/nhg
```

```
    sum `test' [iw=peso`i'] if hg==0, mean
    scalar sp`i'_`test'=1-(r(mean)*r(sum_w)/nonhg)
    sum hg [iw=peso`i'] if `test'==1, mean
    scalar p`i'_`test'=r(mean)
      global rlist "$rlist se`i'_`test'=r(s`i'_`test') sp`i'_`test'=r(sp`i'_`test')
p`i'_`test'=r(p`i'_`test')"
}
}
end

drop _all
use sens
```

Code used to calculate confidence intervals of sensitivity, specificity and PPV after estimating screening tests results and disease status in women with imcomplete screening or women not fully evaluated.

```
prog bootests
version 8.0
tempname sim
postfile `sim' $rlist0 using results3a, replace
set seed 123456
quietly {
forvalues i =1/10 {
        u simul,clear
        bsample
        cal_eff4
        post `sim' $rlist1
}
}
postclose `sim'
end
```